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Final

TECHNICAL EVALUATION OF FISH METHODS IN ENVIRONMENTAL MONITORING FOR THE MINING INDUSTRY IN CANADA

PREPARED FOR:

Aquatic Effects Technology
Evaluation (AETE) Program

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FINAL

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AQUATIC EFFECTS TECHNOLOGY EVALUATION PROGRAM

Notice to Readers

Technical Evaluation of Fish Methods in Environmental Monitoring for the Mining Industry in Canada

The Aquatic Effects Technology Evaluation (AETE) program was established to review appropriate technologies for assessing the impacts of mine effluents on the aquatic environment. AETE is a cooperative program between the Canadian mining industry, several federal government departments and a number of provincial governments; it is coordinated by the Canada Centre for Mineral and Energy Technology (CANMET). The program was designed to be of direct benefit to the industry, and to government. Through technical and field evaluations, it identified cost-effective technologies to meet environmental monitoring requirements. The program included three main areas: acute and sublethal toxicity testing, biological monitoring in receiving waters, and water and sediment monitoring.

The technical evaluations were conducted to document certain tools selected by AETE members, and to provide the rationale for doing a field evaluation of the tools or provide specific guidance on field application of a method. In some cases, the technical evaluations included a go/no go recommendation that AETE takes into consideration before a field evaluation of a given method is conducted.

The technical evaluations were published although they do not necessarily reflect the views of the participants in the AETE Program. The technical evaluations should be considered as working documents rather than comprehensive literature reviews. The purpose of the technical evaluations focused on specific monitoring tools. AETE committee members would like to stress that no one single tool can provide all the information required for a full understanding of environmental effects in the aquatic environment.

For more information on the monitoring techniques, the results from their field application and the final recommendations from the program, please consult the AETE Synthesis Report to be published in the spring of 1999.

Any comments concerning the content of this report should be directed to:

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PROGRAMME D'ÉVALUATION DES TECHNIQUES DE MESURE D'IMPACTS EN MILIEU AQUATIQUE

Avis aux lecteurs

Évaluation technique des méthodes de surveillance environnementale applicables au poisson susceptibles d'être utilisées par l'industrie minière canadienne

Le Programme d'évaluation des techniques de mesure d'impacts en milieu aquatique (ÉTIMA) visait à évaluer les différentes méthodes de surveillance des effets des effluents miniers sur les écosystèmes aquatiques. Il est le fruit d'une collaboration entre l'industrie minière du Canada, plusieurs ministères fédéraux et un certain nombre de ministères provinciaux. Sa coordination relève du Centre canadien de la technologie des minéraux et de l'énergie (CANMET). Le programme était conçu pour bénéficier directement aux entreprises minières ainsi qu'aux gouvernements. Par des évaluations techniques et des études de terrain, il a permis d'évaluer et de déterminer, dans une perspective coût-efficacité, les techniques qui permettent de respecter les exigences en matière de surveillance de l'environnement. Le programme comportait les trois grands volets suivants : évaluation de la toxicité aiguë et sublétale, surveillance des effets biologiques des effluents miniers en eaux réceptrices, et surveillance de la qualité de l'eau et des sédiments.

Les évaluations techniques ont été menées dans le but de documenter certains outils de surveillance sélectionnés par les membres d'ÉTIMA et de fournir une justification pour l'évaluation sur le terrain de ces outils ou de fournir des lignes directrices quant à leur application sur le terrain. Dans certains cas, les évaluations techniques pourraient inclure des recommandations relatives à la pertinence d'effectuer une évaluation de terrain que les membres d'ÉTIMA prennent en considération.

Les évaluations techniques sont publiées bien qu'elles ne reflètent pas nécessairement toujours l'opinion des membres d'ÉTIMA. Les évaluations techniques devraient être considérées comme des documents de travail plutôt que des revues de littérature complètes. Les évaluations techniques visent à documenter des outils particuliers de surveillance. Toutefois, les membres d'ÉTIMA tiennent à souligner que tout outil devrait être utilisé conjointement avec d'autres pour permettre d'obtenir l'information requise pour la compréhension intégrale des impacts environnementaux en milieu aquatique.

Pour des renseignements sur l'ensemble des outils de surveillance, les résultats de leur application sur le terrain et les recommandations finales du programme, veuillez consulter le Rapport de synthèse ÉTIMA qui sera publié au printemps 1999.

Les personnes intéressées à faire des commentaires concernant le contenu de ce rapport sont invitées à communiquer avec M^{me} Geneviève Béchard à l'adresse suivante :

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GLOSSARY

Acute toxicity	Mortality that is produced within a short period of exposure, usually 24 to 96 hours. A relatively short-term lethal or sublethal effect, usually defined as occurring within four days for fish and macroinvertebrates and shorter times for smaller organisms.
ALAD	δ-aminolevulinic acid dehydratase; an enzyme which condenses 2 molecules of aminolevulinic acid to form one molecule of porphobilinogen, a precursor of haem rings used in haemoglobin synthesis.
Annuli	The rings caused by variations in the rate of bone or scale growth due to the seasons. Fish ages can determined by counting the annuli on certain bones (e.g., operculum, otolith, cliethrum) or scales.
Antioxidant	Compound that delays the oxidation of substances by molecular oxygen.
Bioindicators	Species whose presence or absence is an indication of an environmental stressor.
Biomonitoring	Systematic determination of the effects on aquatic life, including accumulation of pollutants in tissue, in receiving waters as a result of the discharge of pollutants: (a) by techniques and procedures, including sampling of organisms representative of appropriate levels of the food chain, appropriate to the volume and the physical, chemical, and biological characteristics of the effluent; and, (b) at appropriate frequencies and locations. The direct measurement of changes in the biological status of an environment or effluent based on the use of living organisms as "sensors" or "indicators"; may involve bioassay testing and/or continued evaluations of the number and distribution of individuals or species in an environment.
Depuration	Elimination of material from the digestive tract of an aquatic organism.
Erythrocytes	Red blood cells.
Genotoxic	Damaging to the inherited material of a living organism, i.e., the genes and their component DNA.

Granulocytes	A cell with granule(s) containing cytoplasm.
Granuloma	A mass or nodule of chronically inflamed tissue with granulations that are usually associated with an infective process.
Histology	The science that deals with tissues and their organization into organs.
Histopathology	The study/diagnosis of disease through examination of the microscopic anatomy of tissues.
Humoral antibodies	Antibodies found in normal functioning bodily semi-fluid or fluid (as the blood or lymph)
Hypoxia	A deficiency of oxygen reaching the tissues of the body.
Leucocytes	White blood cells
Littoral zone	The interface region between the land of the draining basin and the open water of lakes; usually the most productive zone of a lake.
Lysozyme	Enzyme in saliva, tears and mucus. It catalyzes the destruction of bacterial cell walls by hydrolysis, and thus has a bactericidal effect.
Metallothionein	An inducible protein, rich in cysteine residues (high in sulphur content), which is produced by various tissues in response to elevated levels of certain metals (Cd, Cu, Ag and Zn).
Neurotoxic	Toxic to the nerves or neural tissue.
PCBs (polychlorinated biphenyls)	A class of about 70 different persistent, man-made, organic chemicals (consisting of carbon, hydrogen and chlorine) which tend to bioaccumulate through the food chain, causing reproductive failure and cancer. A family of chemically inert compounds, having the properties of low flammability, volatility, water insolubility, and high electrical insulation quality. Past applications include use as hydraulic fluids, heat exchange, dielectric fluids, and plasticizers for plastics. They were banned in 1980, except the continued use in existing electrical equipment.
Pelagic zone	The central water column of a lake or similar body of water, excluding the littoral zone.
Perivisceral	Enclosing an internal organ of the body.

Phagocytes	A cell that characteristically engulfs foreign material and consumes debris and foreign bodies.
Poikilotherm	An organism that possesses a body temperature which varies, approximating that of the surroundings (cold-blooded).
Profundal zone	The deepest region of a lake or similar body of water.
Secondary lamellae	Secondary gill filaments.
Shannon-Weaver index	Index for measuring species diversity.
Sublethal toxicity	Causing or able to cause deleterious effects (anatomical, behavioral, physiological, biochemical) within or towards an organism; may ultimately result in death if exposure is prolonged, or if organism is simultaneously/ subsequently exposed to other environmental stressors.
Tissue somatic indices	A ratio of organ/tissue weight to total body weight.
Vitellogenesis	Yolk formation
Xenobiotic	A foreign chemical or material not produced in nature and not normally considered a constitutive component of a specified biological system. This term is usually applied to manufactured chemicals.

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EXECUTIVE SUMMARY

INTRODUCTION

As part of the Aquatic Effects Technology Evaluation (AETE) program, currently available methods for monitoring effects of metal mining activities on fish in Canada were reviewed. Potentially applicable monitoring tools for all levels of biological organization (chemical to community level) were initially selected by the AETE Technical Committee based on their (a) successful use in the field, and (b) cost effectiveness. In this report, these methods are described and critically evaluated for their suitability in a monitoring program for the mining industry based on a review of the literature and several recent case studies. Detailed reviews are provided for the adult fish survey and fish community survey. Metallothionein and histopathological methods are not reviewed in detail in this report because they are the subjects of separate reports prepared for the AETE Technical Committee.

Criteria for the evaluation of monitoring tools included:

- Ecological relevance.
- Ability to indicate exposure to mining-related contaminants.
- "Early warning" capacity.
- Sensitivity, specificity and response time to metals and low pH.
- Technical difficulty and costs of application.
- Availability of consultants/laboratories that provide services required for the methods.

Recommendations on the use of the reviewed assessment methods for monitoring effects of mining discharges on the contamination and health of fish populations and communities were made based on the proposed objectives and format of a mining industry Environmental Effects Monitoring (EEM) program from AQUAMIN (1996), and experience of the Canadian and Swedish Pulp and Paper Industry EEM programs. AQUAMIN is an initiative focussed on the regulatory issues of mining effluent impact. The program's mandate is to determine whether or not the current Metal Mine Liquid Effluent Regulations (MMLER) limits have adequately protected the receiving waters of mine effluents.

EVALUATION OF METHODS

Metals-in-Tissues

Measurements of metal concentrations in fish tissues are indicative (to various degrees) of exposure of a organism to metal-contaminated effluents. Among the methods reviewed, they are the response that is most specific to high environmental metal levels. They are relatively easy and not expensive to apply, and are widely available. Tissue concentrations are related to tissue type, species, individual size (among other factors), and thus these should be standardized or controlled in assessments of metal burdens in fish.

Biomarkers

Although biochemical-level assessment methods (biomarkers) offer the possibility of being specific responses to certain types of contaminants, and of serving as early indicators of higher-level (more ecologically important) effects, these attributes were not generally observed in studies of mining-related effects. In addition, comparatively fast response times of many biomarkers to elevated metal conditions, and non-negligible effects of natural or other confounding factors promote "background" variability that is too high for their use in a program with an annual or multi-year sampling frequency, as is common for a national EEM program.

In studies with higher sampling frequencies and effort, such as investigations of causes of observed ecological effects, certain biomarkers could provide useful information (e.g., enzymes such as delta-aminolevulinic acid dehydratase [ALAD] that are inducible by specific metals; internal ion imbalances that are associated with low pH).

Tissue Pathology

Assessments of gross tissue pathology are not clearly distinct from methods of histopathology. Examinations of size, color, shape and incidence of lesions in relation to environmental metal concentrations were reported for muscle, liver, gonads, skeletal and dermal structures, spleen and gills. Tissue structure is affected by both general and specific stresses. Effects that were most indicative of exposure to metals and most predictive of higher-level effects were skeletal and dermal damage due to Cd and low pH, and gill lesions due to elevated metals and acidity. Like many of the biochemical-level methods though, tissue pathology appears more useful in investigative studies than in routine monitoring.

Organism

Growth, reproduction and condition are commonly measured organism-level variables in fish surveys, and were reviewed in detail. Growth is the change in size (weight or length) with time or age. Growth is relatively inexpensive to measure, unless specialized and costly techniques are required for determining age. Growth is ecologically relevant, and is sensitive to metal contamination and low pH. Growth (i.e., size and age) can usually be measured on samples of fish collected at any time and for any purpose.

Reproduction is usually expressed as reproductive effort, or fecundity or gonad weight relative to body size. Reproduction may be the most sensitive life history variable in fish. Effects on reproduction should be evident within a year, since the reproductive tissue is generally turned over annually. Fecundity and gonad weight are relatively inexpensive to measure. Reproductive effort should be measured prior to the spawning season, when gonads will be large and well-developed.

Condition is weight relative to length (i.e., "fatness"), and can be used as a general index of well-being. Condition is not particularly sensitive, specific or ecologically relevant. However, the costs of measuring weight and length to assess condition of fish collected for other purposes are usually minimal.

Population

Effects on fish populations can be measured directly or indirectly. Direct population surveys measure relative abundances (usually catch per unit effort [CPUE]). Large samples, in terms of numbers of fish and sample sites, are often required. CPUE and other abundance estimates often have high variances. Therefore, fisheries biologists often monitor or estimate changes in abundance indirectly, from growth, reproduction and mortality. An adult fish survey (AFS) adapts that indirect approach to environmental effects monitoring. An AFS is based on growth, reproduction, and age structure (as a surrogate for mortality), and usually includes condition and some tissue-level variables (e.g., liver size). Fewer fish and sample sites are required for an AFS than for a direct population survey based on CPUE. However, sample processing costs will be higher for an AFS because the fish must be dissected, ova counted, and ages determined.

Community

A fish community survey (FCS) is a direct population survey extended to multiple species. Obviously, more fish and often more sample sites will be required for an FCS than for a single species population survey. However, processing costs are often low, since the fish need only be identified and counted.

Adult Fish Survey

AFS can be cost-effective tools for assessing population-level effects provided that:

- The target species is available and abundant enough to provide adequate sample sizes (= availability).
- Exposure to stressors of interest (e.g., metals) differs among sample sites (= exposure).
- Age, growth and reproduction can be measured precisely and accurately, at a reasonable cost (= measurement).

The availability→exposure→measurement sequence can be used to design and evaluate AFS. Many AFS in the Canadian pulp and paper EEM program failed to meet these three criteria, all of which are necessary.

AFS have usually been conducted on intermediate-size or larger finfish such as suckers or perch. However, smaller, less mobile finfish or bivalves may be as suitable or more suitable. The major disadvantage to using small fish is that some species are multiple spawners, producing several to many clutches of mature ova per spawning season. Reproductive investment is almost impossible to estimate for multiple spawners, because the reproductive tissue may be turned over several times in one season. The limited evidence available suggests that multiple spawning may not be common at higher latitudes, in guarding species, and in other species with large ova.

Sample sites used for an AFS must differ in exposure, but should be otherwise similar. Because AFS and specific variables such as age, growth and reproduction are non-specific, responding to many different factors, study designs should eliminate as many confounding factors as possible. Sampling methods and effort will vary among species and sites, but must be adequate to provide sufficient sample sizes (usually ≥20 fish/sex/species/site).

AFS have been used extensively in Canada and Sweden to monitor the effects of pulp and paper mill discharges. However, an AFS approach has rarely been used to assess the effects of mine discharges and other activities, even though the necessary variables have often been measured on samples collected for other purposes (e.g., to measure tissue metals).

An AFS will generally be the most suitable and cost-effective higher-level tool for monitoring the effects of mining activities, and is recommended for a national EEM program. Experience in the pulp and paper EEM program indicates that an AFS will not be suitable for every mine site. Therefore, the pool of target species should be expanded to include smaller finfish and bivalves, and alternatives such as FCS should be considered acceptable where suitable.

Fish Community Survey

FCS can range from simple qualitative surveys of the presence or absence of a few indicator taxa or guilds to more extensive quantitative surveys based on multivariate analyses or summary indices (e.g.,the Index of Biotic Integrity [IBI]). In an FCS, the basic units of replication are sample sites within larger areas differing in exposure, and not individual fish. Sample sizes should be at least 5 sites per area, and 10-20 would be preferable. Consequently, communities of small fish in streams or littoral zones of lakes are usually more suitable and cost-effective than communities of larger fish.

Once fish have been captured, costs are minimal for identifying and counting them. The abundances of each species are the primary variables analyzed; weights and lengths can often be measured for little extra cost. Exposure indicators or tracers (e.g., tissue metals) are rarely measured in FCS.

Quantitative analytical approaches based on multivariate statistics or summary indices are preferred to qualitative approaches. Multivariate statistical approaches are more objective and defensible than summary indices. However, indices can be useful for reporting and management, provided that multivariate analyses indicate that the assumptions made in calculating the indices are reasonable. One common index, the IBI, is often treated as synonymous with FCS. It is not; IBI must be independently derived for each site or region, based on data from many reference sites. The IBI used in the U.S. are often based on richness of taxa with many more species than occur in Canada. Therefore, IBI will not be suitable for individual mine sites in Canada, although IBI could be developed on a regional basis.

FCS, mostly qualitative or semi-quantitative, have been used in the past in Canadian mine monitoring programs. However, FCS are more commonly used for larger-scale regional surveys, often where many stressors or point sources are present. Defensible quantitative FCS would be too costly to conduct at most individual mine sites in a national EEM program.

RECOMMENDATIONS

An EEM program for the mining industry of the type proposed by AQUAMIN (1996) should:

1. Include standardized measurements of concentrations of metals in fish tissue as a means of determining exposure histories of fish and assessing degree of metal contamination for the protection of human health.

2. Delay the use of any of the reviewed biomarkers and tissue pathology assessments for routine monitoring until further field evaluations establish their relationships to more ecologically relevant effects, and their ease of application.

3. Consider certain tools, such as specific inhibitory or inducible enzymes and tissue pathology, as a means of establishing causal relationships in investigative studies.

4. Include some combination of organism- and higher-level tools, depending on site-specific conditions, because they are more relevant, less costly, and more widely available than lower-level variables.

SOMMAIRE

INTRODUCTION

Dans le cadre du Programme d'évaluation des techniques de mesure d'impacts en milieu aquatique (ETIMA), un examen des méthodes couramment utilisées au Canada pour évaluer les effets de l'exploitation minière des métaux sur les poissons au Canada a été réalisé. Le Comité technique de l'ETIMA a d'abord procédé à une sélection des outils de surveillance pouvant être appliqués à tous les niveaux d'organisation biologique (du niveau chimique à l'échelle des communautés) en se fondant sur : a) l'efficacité démontrée de chacun de ces outils sur le terrain; b) le rapport coût-efficacité de chaque outil. Dans le présent rapport, ces méthodes sont décrites et évaluées de façon objective, à la lumière des résultats d'une étude documentaire et de plusieurs études de cas récentes, en fonction de leur utilité relative comme outil de surveillance pour l'industrie minière (les méthodes de dosage des métallothionéines et d'examen histopathologique sont exclues de la présente évaluation, car elles font l'objet de rapports distincts). Les méthodes de relevés applicables aux poissons adultes et aux communautés de poissons sont examinées en détail.

Les critères suivants ont orienté l'évaluation des outils de surveillance :

- pertinence au plan écologique;
- aptitude à mettre en évidence les effets d'une exposition aux contaminants générés par l'exploitation minière;
- capacité d'alerte rapide;
- sensibilité et spécificité des outils et temps de réponse aux métaux et aux faibles valeurs de pH;
- contraintes techniques et coût d'application;
- existence de firmes d'experts-conseils ou de laboratoires capables de fournir les services requis par les méthodes.

Des recommandations concernant l'utilisation des méthodes d'évaluation des effets des effluents miniers sur la contamination et la santé des populations et des communautés de poissons qui ont été examinées ont été formulées en considération des objectifs et du format établis pour un programme de suivi des effets sur l'environnement (SEE) pour l'industrie minière dans le cadre d'AQUAMIN (1996) et des résultats des programmes de SEE réalisés par les industries des pâtes et papiers canadienne et suédoise.

ÉVALUATION VALUATION DES MÉTHODES

Concentrations de métaux dans les tissus

Les concentrations de métaux dans les tissus des poissons reflètent (à des degrés divers) le niveau d'exposition de ces organismes aux effluents contaminés par des métaux. Parmi les diverses méthodes examinées, ce sont les méthodes de dosage des concentrations de métaux dans les tissus qui présentent les réponses les plus spécifiques aux fortes concentrations de métaux dans l'environnement. Ces méthodes sont relativement simples et peu coûteuses à appliquer et sont largement accessibles. Comme les concentrations de métaux dans les tissus varient selon le type de tissu, l'espèce, la taille des poissons (parmi d'autres facteurs), elles doivent être normalisées ou contrôlées lors des évaluations des concentrations de métaux accumulées dans les tissus des poissons.

Marqueurs biologiques

Bien que les méthodes de détermination au niveau biochimique (utilisant des marqueurs biologiques) puissent présenter des réponses spécifiques à certains types de contaminants et servir d'indicateurs rapides d'effets s'exerçant à des niveaux d'organisation supérieurs (plus importants au plan écologique), ces caractéristiques n'ont pas été observées dans la majorité des études des effets des activités minières sur l'environnement. En outre, les temps de réponse relativement rapides de nombreux marqueurs biologiques observés en présence de fortes concentrations de métaux et les effets non négligeables des facteurs de confusion naturels ou d'autre type contribuent à accroître suffisamment la variabilité de fond pour exclure l'utilisation de ces marqueurs dans le cadre de programmes comportant une fréquence d'échantillonnage annuelle ou pluriannuelle, comme c'est le cas pour un grand nombre de programmes de SEE d'envergure nationale.

Pour les études comportant un effort et une fréquence d'échantillonnage plus élevés, telles les enquêtes visant à cerner les causes d'effets écologiques observés, certains marqueurs biologiques peuvent fournir des informations utiles (p. ex. certaines enzymes comme la delta-aminolévulinate déhydratase dont l'activité est induite par des métaux spécifiques, ou des déséquilibres ioniques internes causés par de faibles valeurs de pH).

Pathologie des tissus

Les méthodes utilisées pour l'examen sommaire de la pathologie des tissus ne diffèrent pas véritablement des méthodes d'examen histopathologique. Des études de la taille, de la couleur, de la forme et du nombre de lésions en relation avec les concentrations de métaux présentes dans l'environnement ont été réalisées pour les muscles, le foie, les gonades, le squelette et les structures dermiques, la rate et les branchies. Les facteurs de stress tant généraux que spécifiques ont une incidence sur la structure des tissus. Les effets qui reflétaient le mieux les niveaux d'exposition aux métaux et permettaient le mieux de prévoir les effets détectables à des niveaux d'organisation supérieur étaient les lésions squelettiques et dermiques induites par le Cd et les faibles valeurs de pH ainsi que les lésions des branchies résultant d'une exposition à de fortes concentrations de métaux et à de faibles valeurs de pH. Comme bon nombre des méthodes biochimiques, l'examen pathologique des tissus semble plus utile pour les études approfondies que pour les activités de surveillance courantes.

Organismes

Dans le cadre de la présente évaluation, une attention spéciale a été accordée à trois variables qui sont fréquemment mesurées dans le cadre de relevés de poissons, à savoir la croissance, la reproduction et la condition. La croissance est définie comme le changement de taille (poids et longueur) qui se produit dans le temps (âge). La mesure de la croissance est une opération relativement simple et peu coûteuse, sauf si des méthodes spécialisées et coûteuses doivent être utilisées pour déterminer l'âge. Ce paramètre fournit des indications écologiques pertinentes et est affecté par la contamination par les métaux et les faibles valeurs de pH. La croissance (c.-à-d. taille et âge) peut habituellement être mesurée à partir d'échantillons de poissons capturés en tout temps de l'année et à n'importe quelle fin.

La reproduction est habituellement assimilée à l'effort de reproduction ou exprimée par la fécondité ou le poids des gonades par rapport à la taille du corps. La reproduction est la variable liée au cycle de vie la plus sensible chez le poisson. Les effets sur la reproduction devraient se manifester en moins d'une année, le tissu reproducteur étant habituellement renouvelé chaque année. Il est relativement peu coûteux de mesurer la fécondité et le poids des gonades. L'effort de reproduction doit être mesuré avant la fraye, alors que les gonades sont bien développées.

La condition, définie comme le rapport du poids à la longueur totale (c.-à-d., « adiposité »), peut être utilisée comme un indice général du bien-être. Cette variable n'est pas particulièrement sensible, spécifique ou pertinente au plan écologique. Toutefois, les coûts associés à la mesure du poids et de la longueur de poissons capturés à d'autres fins en vue d'évaluer leur condition sont habituellement négligeables.

Population

Les effets des activités minières sur les populations de poissons peuvent être mesurés directement ou indirectement. Les études directes mesurent les abondances relatives (habituellement exprimées en prises par unité d'effort, ou PPUE). Ce type d'étude exige souvent le prélèvement de grands échantillons (nombre de poissons et de stations d'échantillonnage). Les valeurs de PPUE et les autres estimations de l'abondance présentent souvent des variances élevées. C'est pourquoi les biologistes des pêches préfèrent souvent suivre ou évaluer les fluctuations d'abondance de façon indirecte, en se fondant sur la croissance, la reproduction et la mortalité. Les relevés des poissons adultes (RPA) utilisent cette approche indirecte pour le suivi des effets sur l'environnement. Ces relevés sont fondés sur l'étude de la croissance, de la reproduction et de la structure par âge (en remplacement de la mortalité) et, habituellement, de la condition et de certaines variables intéressant les tissus (p. ex. taille du foie). Le nombre de poissons et de stations d'échantillonnage requis pour un tel inventaire est moindre que pour une étude directe de la population fondée sur le PPUE. En revanche, l'analyse des échantillons prélevés dans le cadre d'un RPA coûte plus cher, car il faut disséquer les poissons, compter les oeufs et déterminer l'âge des poissons.

Communauté

L'étude d'une communauté de poissons (ECP) est une étude directe de la population étendue à plusieurs espèces. De toute évidence, le nombre de poissons et de stations d'échantillonnage est alors plus élevé que pour l'étude d'une seule population. Par contre, le coût du traitement des échantillons est souvent peu élevé, car il suffit d'identifier et de dénombrer les poissons.

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Relevé des poissons adultes

Dans une perspective coût-efficacité, les RPA peuvent être fort utiles pour évaluer les effets de l'activité minière à l'échelle d'une population, mais seulement dans la mesure où les conditions suivantes sont respectées :

- L'espèce étudiée est suffisamment abondante pour fournir des échantillons d'une taille appropriée (=disponibilité).
- Le niveau d'exposition aux facteurs de stress étudiés (p. ex. métaux) varie d'une station d'échantillonnage à une autre (=exposition).
- L'âge, la croissance et la reproduction peuvent être mesurées de façon précise et exacte à un coût raisonnable (=mesure).

La séquence disponibilité→exposition→mesure peut orienter la conception et l'évaluation d'un RPA. Ces trois conditions sont essentielles au succès d'un RPA. Or, de nombreux relevés effectués pour le compte du secteur des pâtes et papiers ne respectaient pas ces trois critères.

La plupart des RPA ont été réalisés avec des poissons de taille moyenne à grande (p. ex. meuniers, perchaudes). Cependant, dans bien des cas, l'utilisation d'espèces de poissons de plus petite taille ou même de bivalves convient tout aussi bien, sinon davantage. Le principal inconvénient lié à l'utilisation de poissons de petite taille réside dans le fait que certaines espèces peuvent effectuer plusieurs pontes au cours d'une même période de fraye. Dans ces conditions, il devient presque impossible d'estimer l'effort de reproduction, le tissu reproducteur des poissons « multifrayes » étant renouvelé plusieurs fois au cours d'une même saison. D'après les quelques données disponibles, il semble que cette stratégie de reproduction (pontes multiples) s'observe rarement parmi les espèces qui vivent à des latitudes plus élevées ou parmi celles qui veillent sur leur progéniture ou produisent des oeufs de grande taille.

Idéalement, les stations d'échantillonnage utilisées pour les RPA doivent présenter des conditions semblables mis à part, évidemment, le degré d'exposition aux facteurs de stress. Comme les RPA et certaines variables comme l'âge, la croissance et la reproduction sont non spécifiques et fluctuent sous l'influence de divers facteurs, les plans d'étude doivent permettre d'éliminer le plus grand nombre de facteurs de confusion possible. Les méthodes et l'effort d'échantillonnage peuvent varier selon l'espèce étudiée et les caractéristiques des stations d'échantillonnage, mais ils doivent permettre d'obtenir des échantillons d'une taille suffisamment grande (habituellement ≥20 poissons/sexe/ espèce/station d'échantillonnage).

Au Canada comme en Suède, on a largement eu recours aux RPA pour surveiller les effets des effluents des usines de pâtes et papiers sur les écosystèmes aquatiques. En revanche, ce type de relevé a été appliqué beaucoup plus rarement au secteur minier, et ce même si les variables requises avaient souvent déjà été mesurées sur des échantillons prélevés à d'autres fins (p. ex. dosage des concentrations de métaux dans les tissus).

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Dans une perspective coût-efficacité, les RPA représentent dans l'ensemble le meilleur outil à des niveaux d'organisation supérieurs pour la surveillance des effets des activités minières sur les écosystèmes aquatiques. C'est la raison pour laquelle cette stratégie est recommandée pour un programme SEE. Toutefois, l'expérience acquise dans le cadre du programme SEE réalisé pour le compte du secteur des pâtes et papiers révèle que cette approche ne convient pas à tous les sites miniers. Il convient donc d'élargir l'éventail des espèces cibles de manière à inclure les espèces de poissons de plus petite taille et les bivalves et d'envisager l'application d'une autre stratégie comme l'étude des poissons à l'échelle de la communauté (ECP) lorsque cette option paraît souhaitable.

Étude de la communauté des poissons

L'ECP peut prendre la forme de simples relevés qualitatifs visant à confirmer la présence ou l'absence de quelques taxons ou guildes indicateurs ou d'études quantitatives approfondies reposant sur des analyses multivariées ou le calcul d'indices synthétiques (p. ex. indice d'intégrité biotique ou IIB). Dans une ECP, les unités de répétition de base sont les stations d'échantillonnage choisies à l'intérieur de zones plus vastes présentant des degrés d'exposition différents aux facteurs de stress, et non pas chaque poisson considéré individuellement. Aux fins d'une telle étude, il faut prévoir au moins 5 stations d'échantillonnage ou, préférablement, 10 à 20 stations. Pour diverses raisons d'ordre pratique et d'efficacité par rapport au coût, il est donc habituellement préférable d'étudier des communautés de petits poissons vivant dans des cours d'eau ou la zone du littoral plutôt que des communautés de poissons de plus grande taille.

Le coût du traitement des échantillons (identification et dénombrement des poissons capturés) est habituellement négligeable. L'abondance de chaque espèce capturée est la principale variable analysée. Pour un faible montant additionnel, il est souvent possible d'obtenir des mesures du poids et de la longueur de chaque poisson. Les indicateurs du niveau d'exposition (p. ex. concentrations de métaux dans les tissus) sont rarement mesurés dans le cadre d'une ECP.

Les approches quantitatives prévoyant la réalisation d'analyse multivariée ou le calcul d'indices synthétiques sont préférables aux approches qualitatives. Les approches fondées sur la réalisation d'analyses multivariées sont plus objectives et plus faciles à défendre que celles prévoyant le calcul d'indices synthétiques. Toutefois, de tels indices peuvent se révéler fort utiles pour la description et la gestion des communautés étudiées si les analyses multivariées confirment la validité des hypothèses sous-tendant leur calcul. Un indice couramment utilisé, l'IIB, est souvent considéré comme synonyme d'ECP, mais à tort, puisque cet indice doit être déduit indépendamment pour chaque station ou zone à l'aide de données recueillies dans de nombreuses stations de référence. Les IIB utilisés aux États-Unis sont souvent fondés sur la richesse de taxons qui comptent beaucoup plus d'espèces qu'au Canada. Le calcul d'IIB ne convient donc pas pour les sites miniers considérés individuellement au Canada. Par contre, cette pratique peut être valide lorsqu'elle est appliquée à l'échelle de toute une région.

Des ECP essentiellement de nature qualitative ou semi-quantitative ont été effectuées dans le passé dans le cadre de programmes de suivi des effets de l'activité minière au Canada.

Toutefois, les ECP sont plus fréquemment utilisées dans le cadre de relevés intéressant une région plus vaste pour évaluer l'incidence de nombreux facteurs de stress ou de nombreuses sources ponctuelles sur l'environnement. En raison de leur coût prohibitif, les ECP quantitatives défendables se révèlent impraticables dans le cadre d'un programme SEE dans la majorité des sites miniers au Canada.

RECOMMANDATIONS

Aux fins de l'application à l'industrie minière d'un programme SEE correspondant au type proposé par AQUAMIN (1996), il convient :

1. de prévoir la prise de mesures normalisées des concentrations de métaux dans les tissus des poissons. De telles données permettent de déterminer les antécédents d'exposition des poissons et d'évaluer le degré de contamination par les métaux à des fins de protection de la santé humaine;

2. de différer l'utilisation de n'importe lequel des marqueurs biologiques évalués dans le cadre de la présente étude et la réalisation d'examens pathologiques à l'échelle tissulaire dans le cadre d'activités de surveillance régulières jusqu'à ce que des évaluations plus approfondies sur le terrain permettent d'établir des liens avec des effets plus importants au plan écologique et d'évaluer le degré de difficulté entourant leur application;

3. d'envisager l'utilisation dans le cadre d'études approfondies de certains outils, comme certaines enzymes dont l'activité est inhibée ou induite par des métaux spécifiques ou l'examen pathologique des tissus, pour établir des relations de cause à effet;

4. de prévoir l'utilisation d'une combinaison d'outils s'appliquant aux organismes ou à des niveaux d'organisation supérieurs adaptés aux conditions propres à chaque site, ces outils fournissant des données plus pertinentes à un moindre coût et étant plus accessibles que les variables intéressant les niveaux d'organisation inférieurs.

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1.0
INTRODUCTION

The Aquatic Effects Technology Evaluation (AETE) program was established to review appropriate technologies for assessing the impacts of mine effluents on the aquatic environment. AETE is a cooperative program between the Canadian mining industry, several federal government departments and a number of provincial governments. It is coordinated by the Canada Centre for Mineral and Energy Technology (CANMET). The program is designed to be of direct benefit to industry and government. It will evaluate and identify cost effective technologies to meet environmental monitoring requirements. The program includes three main areas: acute and sublethal toxicity testing, biological monitoring in receiving waters, and water and sediment monitoring. The program also includes literature-based technical evaluations and a comprehensive field program.

1.1 PROBLEM STATEMENT

As part of the AETE program, currently available fish monitoring methods as selected by the AETE Technical Committee are being evaluated. To this end, a pilot-study was conducted in 1995 (Beak, 1996) and a field evaluation to verify specific hypotheses took place at four sites during 1997. Given the importance of sampling methodology to the success of biomonitoring, the variety of methods, and the different approaches presently available, the Technical Committee decided that a technical evaluation of fish monitoring methods should be carried out to complement the 1997 field evaluation.

1.2 OBJECTIVES

The objective of this technical evaluation is to provide an up-to-date synthesis of methods for monitoring fish in stream habitats and lakes. In 1990, the B.C. Acid Mine Drainage Task Force requested a complete review of the literature available on biological monitoring techniques related to heavy metals in aquatic environments. This review entitled "Literature Review for Biological Monitoring of Heavy Metals in Aquatic Environments" (Munkittrick and Power, 1990) provided a basis for the work requirements of the present evaluation.

The following technical evaluation builds on Munkittrick and Power (1990) by updating information on fish monitoring methods in the context of their use and effectiveness as biomonitoring tools for assessing environmental effects of mining discharge on the health of fish populations and community. In addition, it describes the different approaches available to understand the effects of stressors on fish and their application to the AETE program. Specifically, this evaluation is intended to:

- Describe the applicable monitoring tools (chosen by the AETE Technical Committee) and include an assessment of their applicability to the mining industry.

- Provide a review of the use of fish tissues as indicators of metal exposure.
- Review the use of fish community/population indicators.

1.3 APPROACH

This review summarizes the available literature on the use of fish methods in environmental monitoring for the mining industry of Canada. Using Munkittrick and Power (1990) as a basis, EVS Environment Consultants (EVS) conducted a computerized literature search for the period 1988-97 covering primary (i.e., journal articles) and secondary (e.g., reports published as part of a series, usually by government agencies) literature. We have also incorporated reports from the AQUAMIN database suggested by the AETE Technical Committee. Metallothionein and histopathology were not researched in this review since these topics are covered in separate reports prepared for the AETE. However, brief summaries of these reports are provided. In addition, EVS incorporated important lessons learned from the Canadian pulp and paper environmental effects monitoring (EEM) program. Conclusions and recommendations from the Swedish EEM program are also incorporated.

This technical review provides the AETE with an overview of the fish monitoring methods currently in use. EVS has attempted to provide information on the feasibility of fish monitoring methods by highlighting advantages, disadvantages and limitations of these methods as monitoring tools. We have provided a critical evaluation of different approaches supported by researchers and biomonitoring practitioners in Canada and have provided examples of the use of fish monitoring methods in different environmental monitoring programs (i.e., pulp and paper) and other countries.

EVS has organized this review according to the Terms of Reference provided by AETE with the information from Munkittrick and Power (1990) re-organized to fit the new format.

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2.0
OVERVIEW

Munkittrick and Power (1990) noted that while many techniques are available to detect effects in the receiving environment, we must consider their appropriateness and relevance in different monitoring situations. For example, fish population or community surveys although ecologically relevant may detect effects only after they have already happened (i.e., they are reactive). In contrast, although detection of a biochemical response in an individual fish may not be directly relevant from a population or community perspective it can help identify a potential impact and allow corrective action before adverse effects are observed at higher levels (i.e., they can be proactive). For this reason, selection of appropriate monitoring techniques may require a compromise between the short time span and sensitivity of proactive techniques (e.g., biochemical indicators) and the ecological relevance and importance of reactive techniques (e.g., adult fish surveys [AFS]). In reviewing available fish monitoring techniques in this report, we emphasize this distinction between ecological relevance and specificity.

Stress responses resulting from contaminant exposure are generally expressed at the individual, population or community level and can consist of primary, secondary or tertiary effects (Munkittrick and Power, 1990). Primary responses are very rapid responses which are transitory, short-lived and readily reversible changes. Secondary responses are of longer duration than primary responses but which may still be reversible. Tertiary responses are the least reversible and longest lasting (Munkittrick and McCarty, 1995).

A useful framework for understanding how stress responses are integrated at the individual, population and community levels is presented in Munkittrick and McCarty (1995) and Munkittrick and Power (1990). Figure 1 illustrates how stress responses are not simply translated along a continuum from chemical and biochemical responses at the individual level to changes at the population and community level. Instead, movement between levels only occurs at well defined intersection or integration points where selected responses are translated to high levels. Recognizing that not all responses (especially chemical and biochemical responses) are transferred to higher levels has important implications for the selection of fish monitoring techniques.

Munkittrick (1992) notes that available fish monitoring techniques are generally not well developed for examining and interpreting the health of fish populations exposed to contaminants. It is therefore important to understand the limitations of monitoring techniques since the choice of techniques at differing levels of complexity will directly influence the ability to detect and trace the response, to establish cause and effect, and to predict the consequences and ecological relevance. The limitations of available fish monitoring techniques can be summarized by examining two different approaches to defining an effect or response to a stressor. Contaminant effects in fish are conventionally evaluated using either a bottom-up (reductionist) approach or a top-down (holistic) approach (Figure 1).

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The bottom-up approach illustrated in Figure 1 looks for responses to stressors at the chemical or biochemical level. The advantage of this approach is that fish generally respond rapidly at those levels to stressors, allowing rapid detection of responses. The disadvantage is that the consequence of specific responses within individual fish are often not well understood and may be absent at the population or community levels. Although a bottom-up approach which focuses on biochemical tests and whole organism physiology has the advantage of high specificity, the lack of direct ecological relevance can be regarded as a significant disadvantage. Consequently, biochemical tests and whole organism physiology might best be regarded as early warning indicators of potential for ecologically relevant changes at the population or community level, but not indicators that such changes have already occurred (Munkittrick and McCarty, 1995).

The top-down approach illustrated in Figure 1 recognizes that it may not be cost-effective to detect large numbers of biochemical changes unless these changes can be interpreted at the whole organism, population or community levels. The rationale is that not all biochemical changes will directly influence ecologically relevant factors in individual fish such as growth, reproduction and survival which may be transferred to the population and community levels. Instead the top-down approach advocates a sequencing in the application of monitoring techniques where chemical or biochemical responses are used to interpret changes that have already been observed at the organism, population or community levels. Such an approach recognizes that although it may not be cost-efficient to examine biochemical responses to anticipate whole organism responses and subsequent impacts at the population or community levels, biochemical responses can be effective in explaining observed changes at higher levels. In this way, the top-down approach allows for a more-flexible, effects-oriented approach based on relevant ecological damage in contrast to a bottom-up approach utilizing available biochemical tests which may or may not be relevant (Munkittrick and Power, 1990; Munkittrick, 1992).

Although arguments regarding the most appropriate focus of field assessments of fish health have looked at the advantages and disadvantages of top-down and bottom-up approaches, increasing attention has recently being given to what has been characterized as a middle-out approach (Munkittrick and Dixon, 1989a; Munkittrick and McCarty, 1995). This approach recognizes that although bottom-up and top-down approaches start at opposite ends of a continuum, they generally tend towards the whole organism level in attempting to extrapolate effects observed at the chemical and biochemical level to the community level in the case of the bottom-up approach or in attempting to relate observed population or community responses to contaminant exposure as in the top-down approach. The advantage of focusing on the whole organism is that it may be possible to extrapolate to contaminant exposure at the chemical or biochemical level (middle-down) or to population and possibly community level effects (middle-up).

Munkittrick and McCarty (1995) caution that although the middle-out approach is well accepted, this approach has some limitations. The problem is that it may not be possible to move seamlessly between the bottom-up approach and the top-down approach. Of particular importance are: (a) the uncertainty in linking cause and effect at the whole organism level; and, (b) in extrapolating between whole organism and population and community responses.

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Establishing cause-effect relationships at the whole organism level is complicated by the fact that chemical and biochemical changes measured in an organism can seldom be linked with certainty to individual contaminants in field situations. Munkittrick and McCarty (1995) note that "biological responses which were thought to be chemical-specific were not specific at all, and biological changes could be associated with a variety of stressors, including non-chemical ones." Extrapolation beyond the whole organism level is increasingly difficult because chemical and biochemical changes resulting from contaminant exposure may not be expressed as whole organism changes which are relevant from a population or community perspective. Although chemical and biochemical changes may cause a variety of whole organism responses, only changes in ecologically relevant factors such as growth, reproduction and survival are likely to be transferred from the individual to the population or community levels. Munkittrick and McCarty (1995) characterize this as "funneling", where a range of chemical and biochemical disruptions at the whole organism level are channeled through a narrow window (i.e., integration or intersection points shown in Figure 1) from the individual to the population and community. Despite the obvious limitations of the middle-out approach, it represents a compromise approach with wide applicability and as such should be seriously considered.

Addison (1996) provides a simple framework for categorizing contaminant effects at various levels of biological organization or complexity which has been adopted for the purposes of the literature review provided in this report. His framework is analogous to the framework described by Munkittrick and Power (1990) and Munkittrick and McCarty (1995) in that it provides a means to evaluate available fish monitoring techniques. Figure 2 illustrates the relationship between specificity of biological effects measurements and their ecological relevance at different levels of complexity. Chemical and biochemical measurements are categorized by their low ecological relevance because of the often ill-defined linkage between chemical and biochemical response and changes at the whole organism that will subsequently be expressed at the population and community level (i.e., growth, reproduction and survival). Although they may not be ecologically relevant, it is recognized that chemical and biochemical measurements can still be useful because they respond in a specific, and often rapid, way to a limited range of stressors. In contrast, community changes are (by definition) ecologically relevant but cannot generally be related to specific causes (Addison, 1996).

The important consideration of response time can be illustrated by changing the vertical axis in Figure 2 from specificity to timeliness of response. Timeliness of response is an important consideration in determining the most appropriate approach to monitoring. Chemical and biochemical responses to contaminant exposure are generally rapid (within hours or days) compared to population of community level responses which may only be observed after weeks, months or years (Addison, 1996; Munkittrick and McCarty, 1995; Munkittrick and Power, 1990). In distinguishing between important considerations such as specificity, ecological relevance and timeliness in this way, Addison's (1996) framework provides a context for understanding and evaluating the potential application of fish monitoring techniques reviewed in the following sections. Where possible, we have attempted to present materials in the following sections according to the general categories shown in Figure 2.

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3.0
LITERATURE REVIEW

3.1 CHEMISTRY AND BIOCHEMISTRY

In the hierarchy of biological organization from the molecular to the ecosystem level, physiological processes affecting cellular and subcellular structure and function are generally the earliest responses to environmental stress. Impairment of these processes underlie toxicological responses at the whole organism, population and community levels. Toxic effects at the lowest levels of biological organization are usually simpler and better understood than effects at higher levels (Haux and Förlin, 1988). These attributes make cellular and subcellular alterations potentially useful indicators of exposure to, or effects of, environmental contaminants.

Such indicators, together with histological assessments indicators, are termed biomarkers (Hugget *et al.*, 1992) and are useful if they are: (a) linked to the more ecologically relevant population, community and ecosystem-level effects; (b) causally related to specific environmental conditions; and, (c) quick, easy and inexpensive to measure (Haux and Förlin, 1988; Mayer *et al.*, 1992; Ryan and Hightower, 1994; Depledge *et al.*, 1995). Application is further dependent on field evaluation, and an assessment of all sources of biomarker variability - organism, environmental (including toxicant effects), and methodological (Mayer *et al.*, 1992).

This section provides an overview of chemical (i.e., metal levels in tissues) and biochemical level methods available for assessing effects of environmental metal contamination on fish. Metallothionein and histopathological methods were not reviewed in detail as they are the subject of separate reports prepared for the AETE Technical Committee. Based on a review of relevant literature, monitoring methods suitable for field application are described and evaluated in terms of their potential effectiveness in detecting sublethal responses of fish to mining activities. Table 1 provides a summary evaluation of selected sub-organism (i.e., enzyme biomarkers) level tools. These tools are highly specific, but in and of themselves have low ecological relevance (Figure 2).

3.1.1 Metals in Tissues

The bioavailability of metal contaminants to aquatic organisms is affected by numerous geochemical and biological factors (reviewed by Luoma, 1983; Campbell and Tessier, 1989). Uptake and regulation of a metal is related to the form of the metal and the physiology of the organism (reviewed by Roesijadi and Robinson, 1994). Ecological factors, such as trophic level and feeding ecology are also important. For example, although metals are generally taken up more efficiently from solution than from particle-bound forms, ingestion can be the most important source for organisms that feed on particles because concentrations of most metals are orders of magnitudes greater in sediments than in solution (Simkiss and Mason, 1983; Luoma, 1983, 1989). The accumulation of metals in tissues results from the integration of several processes - chemical uptake and retention; metabolism, complex and storage;

redistribution and compartmentalization to specific tissues; and excretion/depuration - the interactions of which are incompletely understood (Munkittrick and Power, 1990; Roesijadi and Robinson, 1994). Thus, relationships between metal concentrations in tissues and environment can differ substantially among metals, species, and tissues.

Indications of exposure and effects of contaminants have been sought by measuring whole body, tissue, and blood metal concentrations. In fish, studies have largely examined concentrations in specific tissues (Munkittrick and Power, 1990). Muscle, gill, spleen, liver, kidney, gut, bone and brain have been analysed. Variation among tissues in degree of metal accumulation has been widely documented. Liver, kidney and gill appear more indicative than muscle of Cu and Zn contamination. Concentrations of metals in bones, adjusted for variation in growth rate, are reported as relatively conservative and reflective of chronic environmental concentrations. Quantification of metal concentrations in the diets of fish (in addition to waterborne metal levels) can strengthen the relationship between environmental and tissue metal burdens (Cope *et al.*, 1994; Farag *et al.*, 1994).

Metal concentrations in fish blood are not commonly used to assess levels of environmental metal pollution. With the exception of Pb, the relationships of metals such as Cu and Zn in erythrocytes and plasma of freshwater fish and environmental concentrations are not well known (Munkittrick and Power, 1990). Blood Pb levels which reportedly increase in proportion to waterborne Pb concentrations, are easier to determine than whole body Pb levels, and are highly relevant to fish health because of neurotoxic effects (Hodson *et al.*, 1984).

3.1.2 Ion Status

Exposure to metals and low pH conditions associated with acid mine drainage can alter blood and whole body ionic and osmotic regulation in fish (reviewed in Fromm, 1980; Leino and McCormick, 1984; McKeown *et al.*, 1985; Folmar, 1993). Plasma Na, Cl, K and Ca ions are most commonly used as indicators of osmoregulatory function. Concentrations generally decrease in fish subjected to elevated acid and metal levels. Although altered ion concentrations have been associated with increased plasma glucose (indicating mobilization of energy reserves), cessation of feeding, emaciation and growth retardation, ion imbalances are not well-related to population-level effects (Munkittrick and Power, 1990; Mayer *et al.*, 1992). In addition, these observations come largely from controlled laboratory and *in situ* enclosure experiments (Munkittrick and Power, 1990). However, several studies, including field validations, on Na loss in fish exposed to moderately and severely acidic metal contaminated coal mine water (Grippo and Dunson, 1989, 1996a, 1996b) concluded that Na loss was a very effective indicator of acid mine drainage pollution. In general, disruption of ion balances in blood are indicative only of acute metal and acid toxicity; long-term effects do not usually include osmoregulatory impairment (Mayer *et al.*, 1992).

3.1.3 Inhibitory/Inducible Enzymes and other Proteins

A wide variety of enzymes and other proteins have been assessed for use as biomarkers of contaminant exposure and toxicity. Structure and function of these proteins can be altered by direct action of a contaminant on the molecule or its biochemical system (resulting in responses specific to the contaminant), or by action on other cellular components that produce indirect, non-specific responses to stress. Munkittrick and Power (1990) provide a survey of enzymes and other proteins that have been investigated as potential indicators of exposure to and damage caused by metals (Table 1). Included in the list are a number of biomarkers developed for xenobiotic compounds that cause effects also associated with metal toxicity, such as structural damage to complex cellular macromolecules and membranes. They concluded that few of the proteins studied were sufficiently: (a) responsive and directly related to environmental concentrations of metals; and, (b) correlated to organism-level impairment to warrant widespread field application. However, metallothionein, delta-aminolevulinic acid dehydratase (ALAD) and gill Na/K-ATPase activities were considered responsive to exposures of low levels of certain metals, and potentially useful with other indicators for biomonitoring. Mayer *et al.* (1992) also cited these enzymes, and serum transaminases (aspartate and alanine amino transaminase) as the most suitable enzyme biomarkers for metals.

More recent studies have suggested antioxidants, such as superoxide dismutase and catalase (Palace *et al.*, 1993; Rodriguez-Ariza *et al.*, 1993), and certain heat shock proteins (Ryan and Hightower, 1994) could be useful for assessing metal pollution. But like many of the methods reviewed by Munkittrick and Power (1990) and Mayer *et al.* (1992), field evaluations are needed before implementation. As well, numerous modifying factors related to the fish (sex, age, reproductive state, size, species, nutritional state, handling stress level) and the environment (season, temperature, water chemistry, pH, hardness, oxygen, suspended solids, concentrations of xenobiotic compounds) must be controlled to reduce background variability.

The relation between enzyme and other protein induction or inhibition to higher level effects of contaminants can be poor. For example, Schlenk *et al.* (1996) compared several hepatic biomarkers (cytochrome P450 1A, EROD, hepatic metallothionein mRNA, a 30-kDa stress protein) with a series of whole animal, population and community variables (fish health index, abundance, species richness, percentage of tolerant individuals, percentage of tolerant species, Shannon-Weaver diversity) in male and female carp, largemouth bass, bluegill and white crappie from 13 sites. Out of over 100 calculated correlations, only two were significant at $P < 0.05$. The authors recommended the use of multiple-level rather than single-level indicators to assess effects of environmental contamination.

3.1.4 Lipids and Carbohydrates

Exposure of fish to environmental stressors (including metal contaminants) often results in increased energy demands, during which times energy stores such as lipids and glycogen are mobilized. Determination of whole-body or tissue-specific levels of lipids and glycogen (and proteins, which are utilized after depletion of lipids and glycogen) have been used extensively as indicators of physiological energy status. Mayer *et al.* (1992) review their use as non-specific biomarkers of contaminant stress. Glycogen stores generally decrease in fish under acute and chronic exposure to metals. Although very simple to measure and theoretically of direct relevance to higher level effects such as growth and reproduction, they are also influenced by certain modifying factors (diet, reproductive condition, tissue type, season). Lipids levels generally decline with contaminant stress. As with glycogen, lipids are closely coupled to growth and reproduction, but are also responsive to additional factors. Mayer *et al.* (1992) suggest these two variables are most useful as sublethal indicators of long-term stress. However, establishing background (reference) levels was considered difficult.

Several studies have examined plasma glucose concentrations as biomarkers of contaminant stress (Mayer *et al.*, 1992). Acute exposure to metals generally increased plasma levels in fish, whereas chronic exposures produced both increases and decreases. Further research on the response of plasma glucose concentrations to metal contaminants in field conditions is advisable before inclusion of glucose in monitoring programs.

3.1.5 Biochemical Indicators of Growth

Growth rate is widely recognized as an integrative, non-specific and ecologically relevant response by individuals to contaminant stress (Widdows, 1985; Munkittrick and Power, 1990). Because direct measurement of growth is often difficult in field studies, several biochemical methods have been developed to indirectly estimate growth rates of fish. These include assessing energy balance, protein synthesis, RNA:DNA ratios and nitrogen excretion.

Because metal detoxification requires increased protein synthesis for the induction of metal-binding proteins, replacement of degraded enzymes, and repair of damaged cellular structures, less energy is available to an organism for growth and reproduction (Bayne, 1989). This available energy (P), termed *scope for growth* (SFG), is the net difference between energy absorbed from food (A) and energy lost through respiration (R) and excretion (U):

$$P = A - (R + U) \quad .$$

Bayne *et al.* (1985) describe in detail the methods available for measuring all components needed to calculate SFG. Most work on SFG responses to environmental stress (including metal contamination) has been conducted with marine bivalves, although Mayer *et al.* (1992) cited some work on energetics of fish (Warren and Davis, 1967). Mayer *et al.* (1992) feel that SFG has great potential as an *in situ* indicator of toxicant stress. SFG components are not difficult to measure, and have high ecological relevance. They note that among stress indicators, SFG is probably the most developed and rigorously tested under field conditions. As with other non-specific indicators, SFG is affected by a variety of other factors which must be controlled.

Because growth is primarily a result of protein synthesis, estimates of protein synthesis rates in certain tissues have been correlated with whole organism growth rate (Adelman, 1987; Mayer *et al.*, 1992). Protein synthesis rates can been determined by measuring the incorporation of radiolabeled amino acids, primarily in muscle and scales with fish (Mayer *et al.*, 1992). Of the two tissues, measurement with scales (using glycine uptake) appears methodologically simpler (Adelman, 1987). Wilson *et al.* (1996) observed a 36% reduction in whole body protein synthesis and a 73% reduction in whole body growth rate in juvenile rainbow trout exposed to acid and Al for 32 days. Liver protein synthesis was also reduced. Mayer *et al.* (1992) suggest that the rate of glycine uptake in scales is a potentially useful predictor of whole-organism growth, because it is easier to measure than growth rate and has been tested in the laboratory and the field.

The concentration of RNA in rapidly growing cells is positively correlated to the rate of protein synthesis, and thus can be used to indicate organism growth rate (Haines, 1973; Bulow, 1987). RNA concentration is usually stated relative to DNA content. While research in laboratory and field environments has documented some sensitivity of RNA concentration to metal exposure, it has also shown RNA levels to be highly variable and affected by age of the organism, and such environmental factors as food supply, temperature and pH (Mayer *et al.*, 1992). Since the RNA:DNA ratio is easier to measure than whole body growth rate it could be a useful biomarker. However, further field validation studies are required to assess variation due to modifying factors, especially food supply and temperature.

With increased protein utilization under severe levels of environmental stress, nitrogen excretion will increase due to amino acid oxidation, and the ratio of oxygen consumed: nitrogen excreted (O:N) will decrease (Mayer *et al.*, 1992). Although studies examining the use of O:N as a biomarker of metal pollution in fish are lacking (Munkittrick and Power, 1990; Mayer *et al.*, 1992) recommend its measurement whenever scope for growth is assessed.

3.1.6 Biochemical Indicators of Reproduction

Reproductive steroid levels and alterations related to vitellogenesis have been examined as indicators of reproduction in fish (Munkittrick and Power, 1990; Mayer et al,. 1992). Steroids, such as testosterone, 11-ketotestosterone, estradiol and progesterone, are well known to be affected by xenobiotic compounds, but only a few reports (Mount, 1988; Thomas, 1988) discuss effects of metals (confounded with low pH or xenobiotics) on reproductive steroids. Similarly, information on direct effects of metals on vitellogenin and related variables in field environments is lacking.

3.1.7 Biochemical Indicators of Immunity

The use of immunological biomarkers to assess environmental stress is reviewed by Weeks *et al.* (1992). Immune function is mediated by leucocytes via two pathways:

1. Non-specific immune responses mediated by mononuclear phagocytes and granulocytes.

2. Specific immune responses mediated by effector leucocytes (such as lymphocytes) which are directed against, and are specific for, eliciting agents (antigens) (Weeks *et al.*, 1992).

Non-specific assays suitable for assessing fish health and other responses to contaminants include leucocyte volume, blood differential counts, lysozyme levels, macrophage function, and humoral antibody assays. Although immune function is altered by a broad range of contaminants, the vast majority appear to be xenobiotics. Only a few examples of the use of fish immunological responses to metal exposures were cited by Weeks *et al.* (1992): (a) certain concentrations of cadmium reportedly stimulated humoral antibody production in cunner, *Tautogalabrus adsperus*, but suppressed it in striped bass, *Morone saxatilis* (Robohm, 1986); and, (b) heavy metals suppressed antibodies in brown trout, *Salmo trutta* (O'Neill, 1981).

Immunological processes are capable of rapid and integrative responses to low concentrations of toxicants, can be reliably tested, and have been used with many fish species. They are thus potentially useful biomarkers. Unfortunately, application for indicating metal contamination is poorly developed.

3.1.8 Biochemical Indicators of Respiration

Respiratory disfunction in fish exposed to elevated metal and acid levels appears to arise from the impairment of gill function (Klaverkamp, 1982). Hypoxia can occur due to increased mucous secretion, depression of aerobic processes, increased coughing frequency, loss of oxygen sensory ability, skeletal muscle paralysis and vasoconstriction in the secondary lamellae (Munkittrick and Power, 1990). In fish, respiratory responses to metal contaminants are commonly not evident until acutely toxic concentrations are reached (Klaverkamp, 1982). Thus indicators of respiratory stress are likely not useful as biomarkers of environmental metal levels.

3.1.9 Genotoxic Responses

The assessment of damage to DNA has been proposed as a means of detecting genotoxic effects of environmental pollutants. Shugart *et al.* (1992) reviewed current methods available for monitoring DNA damage. These include:

1. Direct measurements of DNA structural damage, such as DNA adducts, strand breakage and changes in minor base composition.

2. Measurements of DNA repair (unscheduled DNA synthesis).

3. Detection of irreversible events in the genome of the exposed organism, such as cytogenetic effects (chromosome analysis, sister-chromatid exchange, micronucleus assay), abnormal DNA distribution (as measured by flow cytometry), and mutations (oncogene activation, mutation rates).

Shugart *et al.* (1992) noted that genotoxicity is a stage in a series of events that may, or may not, lead to higher level damage. Monitoring methods are in various levels of development. Because metals are not generally considered carcinogenic or mutagenic, these methods do not appear applicable for assessing effects of mining activities.

3.1.10 Other: Metallothionein

The evaluation of metallothionein (MT) as a biomarker for effects of metal-contaminated effluents on aquatic biota was the subject of a separate report by Couillard and St-Cyr (1997). For completeness and comparison, key findings from that report are presented below.

Published field evaluations strongly indicate that MT responds in a dose-dependent manner to ambient concentrations of metals or groups of metals (in particular, to Cd, Cu, Zn, Ag). There is some evidence of a relationship between level of MT and "health" of aquatic organisms. Only one study found that MT served as an "early warning" indicator of higher-level effects. Non-toxicological factors also affect MT levels in biota, and these require further study. Reliable methods for MT detection and quantification exist, but are not widely available. The authors (Couillard and St-Cyr, 1997) conclude that:

- MT is a useful biomarker of exposure to certain metals.

- MT quantification is useful only with measurements of other biotic and abiotic variables.

- Additional studies on the role of MT in metal toxicity are required.

- MT as an "early warning" indicator is not established.
- Standardization of MT protocols for sampling and analyses is required for a national EEM program.

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3.2 TISSUE-LEVEL TOOLS

This section discusses tissue-level indicators, referred to as individual integrators by Munkittrick and Power (1990). As exposure to contaminants can result in the swelling or atrophy of fish tissues, in theory it should be possible to use tissues as indicators of metal contamination. Munkittrick and Power (1990) recommend that morphological parameters be recorded and monitored when other fish population data are collected. In addition, they suggest that tissue somatic indices are valuable surveillance tools which can be easily monitored. However, few studies have collected sufficient data to evaluate the usefulness of tissue-level tools.

Results are often contradictory. For example, a necropsy assessment conducted during a study on fish in the Clark Fork River, Montana (Farag *et al.*, 1994), found that the appearance of vital organ systems were unaffected by metal exposure via water or diet. However, with respect to metal bioaccumulation in fish tissues, Miller *et al.* (1992) found that the liver and kidney were reliable indicators of chronic environmental Cu and Zn contamination, and Zn exposure was also reflected in bone tissue. Muscle was found to be a poor indicator of low-level Cu and Zn contamination.

Prior to 1984, few comprehensive studies indicated strong correlations between increased neoplasm frequency and increased chemical levels in sediment (Malins *et al.*, 1984). Although a number of studies have shown the existence of anomalies on the liver, gill, and kidney of fish exposed to organic contaminants such as PCBs (Adams *et al.*, 1993), there is limited information regarding the specific effects of metals on the appearance of major tissues or organs. A study conducted by Black *et al.* (1982) relates the presence of Cu mining wastes in Torch Lake to the presence of tumors and lesions. Farag *et al.* (1995) conducted necropsy measurements on brown trout (*Salmo trutta*) exposed to metals (As, Cd, Cu, Pb, and Zn) in the Clark Fork River, Montana. They noted little difference in parameters measured for brown trout in reference and exposure sites even though Cu, As, Cd, and Pb tissue residues were significantly higher than in trout from reference areas.

The AQUAMIN Final Report did not contain information linking tissue metal concentrations to the health of fish tissues/organs. However, the report summarized observations from studies showing increased tissue metal concentrations in fish exposed to mining effluent (AQUAMIN, 1996).

The following section discusses specific fish tissues/organs on an individual basis and attempts to provide some detailed information regarding the ecological relevance of the use of tissue level tools in field situations. Due to the limited literature available, there is little information with which to judge the usefulness of these parameters. In general, these tools are intermediate in terms of specificity and relevance (Figure 2).

3.2.1 Liver

The normal fish liver condition is generally considered to be solid red to light red in colour but is dependent upon both species and reproductive state. Abnormal manifestations of the liver include: fatty and light tan in colour; liver nodules; focal discolouration; and general discolouration (Goede, 1993). In addition, liver enlargements may occur. Possible causes of enlargement are a high carbohydrate diet (Dixon and Hilton, 1981) or (indirectly) increased enzyme activity for detoxification of compounds (Dixon *et al.*, 1987). Liver enlargements can be detected through the use of the hepatosomatic index (HSI) which expresses the liver weight as a proportion of the total body weight.

Black *et al.* (1982) found saugers and walleyes exposed to Cu mining wastes (Cu, Pb and Zn) in Michigan were commonly affected with hepato-cellular carcinomas. The most striking finding involved the livers of the saugers, which exhibited gross signs of neoplastic disease. The livers ranged in colour from pale yellow to a normal reddish tan and contained raised nodules and multiple subcapsular, fluid filled cysts. The nodules ranged from single foci to large raised neoplastic-appearing nodules.

3.2.2 Gonads

Gonadal atrophy can arise from food limitation or reproductive dysfunction. Atrophy can be detected by expressing the gonadal weight as a proportion of the total body weight to give a gonadosomatic index (GSI). Friedmann *et al.* (1996) conducted a study on the effects of mercury on gonadal function in Lake Champlain Northern Pike (*Esox lucius*). No significant correlations were found between Hg content and the GSI. However, Dey and Bhattacharya (1989) demonstrated a decrease in GSI in a species of snakehead fish *(Channa punctatus)* exposed to 16.7 μg/L Hg.

Other possible signs of gonad exposure to metals include lipogranuloma-like lesions (Black *et al.*, 1982), that consist of macrophage-like cells containing a yellow crystalloid-appearing material. In addition, the authors noted that a number of Torch Lake saugers exposed to mining waste had atrophic gonads. However, a study conducted in a Cu (13-15 μg/L) and Zn (209-253 μg/L) contaminated lake (Munkittrick and Dixon, 1988) showed no detectable effects on gonadal development measured morphologically.

3.2.3 Skeletal and Dermal Effects

A number of factors can lead to damage in the spinal column of fish, including congenital defects (some induced by contaminants), parasitic infections, electrical shock and direct or indirect contaminant toxicity (NRCC, 1985). Skeletal anomalies include deformed fins, the lack of one or more fins and pelvic girdle, pugheadedness, asymmetric cranium, shortened operculae, fused and deformed vertebrae and spinal curvatures (Sloof, 1982). Spinal pathology has been advocated as a useful indicator of contaminant effects on fish because spinal curvature in fish often increases with increasing proximity to sources of pollution. Mehrle *et al.* (1982) demonstrated a strong correlation between measures of vertebral strength in east-coast striped bass and tissue levels of organochlorine compounds, proximity to sources of pollution, prevalence of spinal curvature and reductions in population size.

Spinal column damage caused by chemicals in the aquatic environment has been reported by Dawson (1964, 1966, 1971) and Imada and Yoshizumi (1973). Muramota (1981a) documented vertebral malformation in fish induced by exposure to Cd where exposed fish suffered decalcification from the bones. This response indicates that the chronic toxicity of Cd induces abnormal bone tissue together with the escape of Ca from the bones leading to cavitation, shortening and assimilation of cartilage. Muramota (1981b) also documented vertebral column damage and decrease of calcium concentration in fish exposed to Cd in the laboratory. Cd concentrations in the spinal column of deformed fish were found to be significantly higher than in those of normal fish. Muramoto (1981b) found that Cd was present at higher levels in the viscera, gills and vertebrae of fish experimentally exposed to Cd than in the control fish. Marked differences in Cd concentrations were found between the viscera and gills of skeletally deformed fish and those of normal fish.

3.2.4 Spleen Size

According to Fish Health/Condition Assessment Procedures (Goede, 1993), black, red and granular spleens are all considered to be normal manifestations of spleen condition. Other manifestations of spleen condition such as enlarged tissue, nodules, and grey mottling can be due to contaminant exposure. Black *et al.* (1982) found that fish exposed to mining waste containing Cu, Pb and Zn in Torch Lake, Michigan were commonly affected with perivisceral masses resembling mesotheliomas that were usually associated with the mesenteric capsule of the spleen, but sometimes appeared to be attached to mesenteric fat. These neoplasms varied in size and colour. Sizes ranged from large polypoid growths (4-6 cm in diameter). Colours ranged from an opaque tan through a translucent pinkish-tan to a colourless, somewhat hyaline appearance.

3.2.5 Gills

Gills are likely the primary initial target of metal/H+ toxicity and cytological changes in gill morphology in fish may occur as a result of contaminant exposure. For example, Johnson and Bergman (1984) found that gill lesions occurred in all organisms exposed at contaminant concentrations above a threshold level. Impacts of metals and acid on fish gill lamellae include a wide variety of changes including hyperplasia (increased number of cells) and hypertrophy (increased cell size measured as cell height and volume) of mucous cells, separation of the basilar membrane, and necrosis and fusion of secondary lamellae (Mitz and Giesy, 1985, Versteeg and Giesy, 1986). Munkittrick and Power (1990) recommend that measurements of gill histological changes are valuable since they have been linked to changes at the organism level.

3.2.6 Other: Histopathological Assessments

A separate report was recently produced that evaluated the suitability of histopathological methods for monitoring effects of mining activity (GlobalTox, 1997). Because of the close relationship (and similarity of tissue "pathology" and histopathology), the findings of this evaluation are summarized:

- The training of pathologists and morphologists is important for the standardization of data collection and interpretation.

- The predictive value of histopathology in determining "health" varies from good to excellent.

- The use of histopathology as a monitoring method is limited by (a) need for multiple samples through time, (b) sample preservation, (c) lack of specificity of response and confounding factors, and (d) cost effectiveness.

The authors recommended that these methods were:

- Useful for the diagnosis of causes of fish die-offs.

- Indicative of fish health.

- In need of supporting abiotic and biotic variables.

As a tool for an EEM program, it was felt that the methods require further evaluation.

3.3 ORGANISM

This section discusses measures made at the whole-organism level. The measures or tools are referred to as individual integrators (which also include tissue-level measures) by Munkittrick and Power (1990), and as indices of well-being by Shuter (1990). Reproduction, growth and condition are also used in population-level assessments (Section 3.4). Table 2 provides a summary evaluation of organism- and higher-level tools. These tools are intermediate in terms of specificity and relevance (Figure 2).

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3.3.1 Growth: Size-at-Age

Growth is the change in size (weight or length) with time. Growth is expressed as an increment (change in size over some fixed interval), growth rate (change in size per unit time), or as an equation relating size to age. In general, growth rates will not be constant (i.e., linear relationship between size and age) over the lifespan of fish. If growth equations are compared (e.g., in ANCOVA), or if sizes at one or more ages are compared (e.g., in ANOVA), those are comparisons of size-at-age rather than comparisons of growth rates.

There are two types of growth rates or equations (Ricker, 1975):

- True (individual) growth
- Apparent (population) growth

True growth, or the growth of single individuals, can be estimated by measuring size on the same individuals at several different times (usually impossible in the field) or by back-calculating size-at-age from scales or other structures used to determine age (Ricker, 1975, 1992).

Back-calculation involves measuring the distance between successive annuli on the age structure, then converting those distances to growth increments, based on the relationship between the size of the age structure and body size. Back-calculated sizes-at-age can be estimated from a single sample of fish. In many cases, conversion of growth increments for the age structure to increments for body size is unnecessary. The analysis can focus on growth of the structure itself.

Gagnon *et al.* (1995) provide an example of the use of back-calculated growth in environmental effects monitoring. Juvenile sizes (i.e., at ages 2, 3, and 4 years) of white suckers (*Catostomus commersoni*) were estimated and compared between a reference river and a river receiving pulp mill effluent. The authors compared radii of the age structure (pectoral fin rays) without converting those radii to body sizes. The relationship between fin ray radius and body length was relatively weak, which reduced the effectiveness of comparisons of back-calculated size-at-age.

Population growth can be measured by calculating sizes at various ages from a single sample of fish, or by calculating sizes of a single cohort (year-class) sampled annually (Ricker, 1975). Population growth will not represent individual growth if mortality or sampling is size-selective. For example, if mortality is higher for larger fish, population size-age relationships (i.e., growth equations) based on a single sample or repeated sampling of a single cohort will underestimate individual growth (=Rosa Lee's phenomenon). Lee's phenomenon can cause problems for some calculations used in fisheries management. The standard assumption in impact or effects assessment (rarely stated or tested) is that size-selective mortality is similar for reference and exposed fish, and that any differences in population growth therefore reflect differences in individual growth.

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If growth is used only as an index of individual well-being, rather than as part of a population-level assessment, it is preferable to measure individual growth, as well as or instead of, population growth (i.e., to eliminate any effects of size-selective mortality). Juvenile growth is usually superior to adult growth for effects assessment at the individual or organism level. First, adults devote energy to reproduction as well as growth, and fecundity and gonad size are usually positively related to body size. Therefore, growth differences could be either positively or negatively correlated with differences in reproductive effort. Juveniles grow more rapidly than adults, because they are not devoting energy to reproduction as well as growth. Ages of juveniles are usually easier to determine and subject to less measurement error. Growth effects can also be identified more rapidly in juveniles than in adults, especially in populations surveyed shortly after mine operation begins.

Figure 3 illustrates the above points using population growth for unexposed mature male and female white suckers from an Alberta river which receives a pulp mill discharge. Both sexes mature at about age 5 years. By age 5 years, males reach 80 to >90% of maximum body weight. Therefore, most of the growth occurs prior to maturity (i.e., in juveniles), and effects on adult male growth would be difficult or impossible to detect. Also, adult male growth may be an unimportant variable, since there appears to be little or no selection pressure for increased male body size. Even though females continue to grow after maturity, presumably because of selection pressure for increased reproductive output (i.e., fecundity), weight at age 4 - 5 years (i.e., juvenile growth) is equal to the subsequent increase in weight over the next 10 years. For this population, mild enrichment effects (i.e., increases) were observed for both reproduction and growth of exposed females. However, for males, the enrichment effect was observed for reproduction but not growth. Finally, none of the mature adult suckers had been exposed during their entire juvenile growth period, since the mill began discharging in late 1990 and the most recent sampling was in 1994. Eventually, effects on juvenile growth would be evident from vertical displacement of the growth curve (circles and lines) in Figure 3, but those effects may not be evident until the next century. A better assessment of effects on juvenile growth would come from sampling juveniles or from back-calculating juvenile growth of the younger adults.

Growth is an important and relevant parameter for fish, which integrates many effects at lower levels (Table 2). Body size is probably the most important biological variable at the organism level. Many other variables are correlated with body size, especially in poikilotherms (Peters, 1983). Weights and lengths are easily and rapidly measured on fish, so the major costs of examining population growth are associated with collecting the fish, and sometimes with ageing them. Costs for age determinations are ≤$10/fish when scales or opercula are used, but may be more when fin rays or otoliths are used. Estimating individual growth from back-calculation is more labour intensive. Costs can be reduced, and precision increased, by back-calculating growth increments for one or a few intervals >1 year. For example, growth increments could be calculated for age 0 to minimum age at maturity for an analysis of juvenile growth.

As numerous laboratory studies have shown (Suter *et al.*, 1987), metals affect fish growth, usually negatively. As with any integrative and relatively non-specific parameter, growth is also affected by many other factors, especially in the field. Metals may also affect fish growth indirectly, as well as directly, by affecting their prey (usually benthic macroinvertebrates or zooplankton) (e.g., Munkittrick *et al.*, 1991). In adults, growth and reproductive effects may be confounded or difficult to distinguish. Growth, or size-at-age, reflects cumulative or long-term effects, so growth effects on fish, especially adults, will generally not be evident until several years after some intervention (Sandström, 1996). Growth is rarely a rapid-response measure, except for juveniles.

Examples of relevant field studies using growth (and reproduction) are considered in Section 4.2, since growth is generally measured as part of population-level assessments. Noakes and Campbell (1992) provide an interesting example of a study in which growth of geoduck clams (*Panope abrupta*) was back-calculated to assess impacts from various sources. Since the clams can live for more than 100 years, the authors were able to reconstruct annual growth increments for most of the 20th century and apply time series analyses. They observed an increase in annual growth increments (by ~8%) in 1919, coincident with an increase in temperature, and an even larger decrease (~20%) after 1960, soon after log storage and booming were initiated at the study site.

Methods for measuring and analyzing growth are provided in Ricker (1975) and in standard fisheries biology texts (e.g., Nielson and Johnson, 1989; Schreck and Moyle, 1990). Growth is recommended for inclusion in environmental monitoring programs for metal mines simply because it is part of population-level assessments. Growth should also be included in effects assessments at lower levels, since costs for measuring growth are generally low once the fish have been captured for other purposes. In either population- or lower-level studies, investigators should consider measuring individual as well as population growth, and should consider estimating juvenile growth by sampling juveniles or by back-calculation. In long-lived species, back-calculated growth can provide time series for retrospective impact assessment.

3.3.2 Reproduction (Reproductive Effort)

At the individual or population level, reproductive effects are usually examined by measuring reproductive effort (i.e., fecundity or gonad weight, usually relative to body size). Since most fish produce large numbers of young which subsequently suffer high mortality, it would be better to measure the number of viable young at some early life stage rather than simply the number of ova produced. However, estimating the number of viable young produced by an individual female is impossible in field studies.

In field studies, reproduction is usually measured by collecting a sample of adults, removing and weighing the gonads, and in the case of females, counting the ova in one or both ovaries, or in a subsample of known weight. Gonad weights are typically given as a percentage of body weight (= Gonadosomatic Index or GSI). However, gonad weight and fecundity should be analyzed in ANCOVA, with body weight or length as the covariate (i.e., *x* variable). Refinements can include measurement of the dry weight or energy content of ova, rather than simply wet weight, and measurement of various sub-organism reproductive parameters (e.g., hormone levels).

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The basic measurements of gonad weight and fecundity are relatively inexpensive, so most of the cost is associated with sample collection. Sample timing is important since the fish should be collected when gametes, especially ova, are reasonably well developed. Reproductive effort can be difficult to estimate or analyze in multiple or batch spawners (i.e., species, usually small, which mature several clutches per year) (Heins and Rabito, 1986; Burt *et al.*, 1988; Paine, 1990), and in fish which "skip" reproductive years (i.e., do not reproduce every year).

Reproductive effort is sensitive to metals and other contaminants, and is a critical component of fitness at the individual or population level (Suter *et al.*, 1987; Barnthouse *et al.*, 1987, 1989, 1990). Like growth, reproductive effort is an integrative and non-specific parameter. In fact, reproduction and growth are really two forms of a single measure - energy expenditure (Gibbons and Munkittrick, 1994). Therefore, reproductive effects measured in isolation may be correlated with unmeasured growth effects. The key difference between the two measures is that reproductive tissue is usually turned over annually, so that reproductive effort responds more rapidly to contaminants and other anthropogenic effects. Reproductive effects can be detected in shorter intervals, usually ≤ 1 year. For the white suckers in Figure 3, reproduction is probably superior to growth as an effects measure since the mass of reproductive tissue produced annually is greater than the annual gain in weight, especially for males.

Gonad size and fecundity are simple and inexpensive to measure. Major concerns include collection of sufficient numbers of mature fish when gonad development is similarly advanced, and knowledge of basic biology of the study species. Like growth, reproductive effort can and should be measured for little or no cost when fish are collected in the field for measuring other, usually sub-organism, parameters. EVS, ESP and JWEL (1997) cite costs of $60-75/fish for fecundity estimates. Reproductive effort may be more difficult to measure and/or less useful in field studies than growth, because only mature fish can be used, the sexes must be separated, and there is no way to back-calculate past reproductive effort. Reproductive effort is also more difficult to measure than growth in the laboratory because longer-term (full- or partial life cycle) tests and/or larger holding facilities are required for mature adults.

3.3.3 Condition

Condition is defined as weight relative to length (="fatness" or robustness), and is an indicator of short-term energy storage. Condition is usually expressed as an index (*k*):

$$k = 100(weight)/(length)^3,$$

where weight is in g and length in cm. k=1-2 for most fish.

Fisheries biologists have recently begun to use relative weight (W_r) as an index of condition. Liao *et al.* (1995) discuss the calculation and application of W_r. An overall or standard weight-length regression is developed from literature data on many populations. Then W_r is calculated as the weight of a captured fish divided by the weight predicted for a fish of that length by the standard regression. A fish with $W_r \approx 1$ will therefore be "normal" for that species. Development of the standard weight regression can be time-consuming unless a regression is already available from the literature.

Cone (1989) reviews several other condition indices. He recommended that statistical analyses of condition should be based on comparisons of weight-length regressions in ANCOVA, and not on comparisons of k and other indices in ANOVA (see also EC/DFO, 1995). However, the indices can be useful for presentation and summary, provided that the analyses indicate that the assumptions made when the indices are compared have not been violated.

Measuring condition is trivial since weights and lengths of fish are routinely measured in field and laboratory studies. As with growth and reproduction, the major costs are associated with capturing the fish. Investigators should carefully consider which measure of body weight is appropriate. Carcass weight (gonads and digestive tract removed) is preferred, especially if reproductive effort is also analyzed. Dry weight would be a better measure than wet weight, since the latter is affected by water content, but obtaining dry weights of large fish in the field is impractical.

As a general stress indicator, condition can be affected by many factors other than contaminants. In fact, condition is arguably a better tool for assessing the impacts of non-contaminant stress (e.g., gas supersaturation; habitat alteration) than for assessing contaminant impacts. Liao *et al.* (1995) found that W_r of two species differed significantly among Quebec lakes, and that lake means were correlated with benthic invertebrate biomass (i.e., prey availability). There was no relationship between condition and growth, despite largely anecdotal evidence for such a relationship in the literature. In impact assessments, there is usually a relationship between condition and either growth in length or weight (Gibbons and Munkittrick, 1994). In fact, condition or the weight-length relationship can be calculated from regressions of weight and length on age (i.e., the relationship between Y and Z can be calculated from the relationships of both with X).

SOMMAIRE

Condition is not as relevant biologically as either reproduction or growth, and is probably too non-specific to be recommended as a stand-alone tool for assessing impacts from metal mines. However, weight and length data are or should be available from any field study, so condition should always be calculated and compared in effects monitoring. Because measurement error for weight or length is generally less than measurement error for reproductive effort or age, condition usually provides a more powerful test for effects for the same sample size (Sandström, 1996). Condition also responds more rapidly to stress than do growth or reproduction, although effects may be transient or may eventually be translated into effects on reproduction or growth (Gibbons and Munkittrick, 1994). In other words, energy may be stored as body fat (lipids) in the short term, but that stored energy may eventually be converted to skeletal growth (i.e., growth in length) or reproductive tissue.

3.4 POPULATION

Population status and trends can be assessed directly, by measuring abundances of one or more ages or stages, or indirectly by measuring reproductive effort, growth and age structure/survival. Both approaches can be combined, and often are, in fisheries management. For a single species, population-level effects are ultimately the only relevant effects. If effects at lower levels are not correlated with reductions in abundance then those lower-level effects are arguably unimportant (but see Humphrey *et al.* [1995] for an alternative viewpoint).

Table 2 provides a summary evaluation of population-level tools. These tools are highly relevant, but relatively non-specific (i.e., population abundances respond to many factors other than mining activities). Section 4.2 discusses the application of indirect measures of population status (i.e., the adult fish survey [AFS] approach) to metal mine environmental monitoring in detail because the AFS was one tool selected by the AETE for future use. Smith (1994) provides a good historical review of population-level assessments and tools over the last century. Getz and Haight (1989) review the development of population models and the application of those models to exploited fish populations. Barnthouse *et al.* (1987, 1989, 1990) provide good examples of the use of population models to estimate risks from contaminants, and discuss the rationale for using indirect measures. MacDonald *et al.* (1991) review methods for, and usefulness of, population-level assessments for monitoring streams in the Pacific Northwest. Their manual focuses on the effects of forest harvest, but also considers effects from hardrock mining, placer mining, and road construction.

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3.4.1 Direct Measures (CPUE)

Direct measurement of population abundances requires samples of one or more life stages. Because absolute abundances are difficult to estimate, abundance indices (i.e., CPUE) are often used. Fisheries biologists usually follow abundances of single populations over several to many years (i.e., trend monitoring or temporal design). This approach was used in all case studies provided in Shuter (1990). Environmental effects monitoring usually involves comparison of reference and exposed populations at one or a few times. We did not find any recent studies in the primary literature which used that approach to directly measure population-level effects of mines on fish. However, the reference versus exposed (i.e., spatial) design has been used to examine effects of other anthropogenic activities on fish. For example, Carline and Jobsis (1993) assessed the effects of aerial emissions of metals from a smelter on stream trout densities. More generally, any study comparing fish communities between reference and exposed areas could be considered a set of single-species population studies.

Sampling gear and methods are of key importance in direct measurement of population abundances. EC/DFO (1993) provides recent reviews of capture gear; several chapters in Hocutt and Stauffer (1980) review and discuss capture methods and methods of estimating abundances. Local fisheries biologists, bait fishermen, and museum personnel can also provide information on the best capture methods for specific species. Ricker (1975) is the standard source on techniques for estimating abundances and their confidence limits or variances. Mahon (1980), Paller (1995) and Jones and Stockwell (1995) discuss abundance estimates from electrofishing in streams, which would probably be the approach used in most mining environmental monitoring studies. Lyons (1986) discusses the use of beach seines in lakes; Hamley (1975, 1980) reviews the use of gillnets; Craig *et al.* (1986) provide estimates of variability for gillnet catches from a baseline lake survey in the Alberta oil sands area. There are many other applicable references, and methods are also provided in most population- or community-level assessments cited in this report.

The major disadvantages of direct population-level assessments are that large samples are often required and that abundance estimates or CPUE have large variances. Fisheries biologists usually work with exploited populations so the commercial or recreational catch often provides most of the data for abundance estimates. Normally, exploited species are not recommended for use in environmental monitoring programs because exploitation is a confounding factor. However, Harris (1995) discusses several approaches which could be used to assess environmental impacts on commercial or recreational fish species, using data routinely collected for the fisheries.

The effects of destructive sampling (e.g., by gillnet) can be as great as or greater than those from mining or other anthropogenic activities. Destructive sampling in lakes used for recreational fishing is also unpopular with local fishermen and area residents.

Small species or juveniles of larger species will be more abundant, and require less sampling effort, than adults of large species. Smaller fish can also be sampled over smaller spatial scales, which will generally reduce field costs.

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Coefficients of variation (CV) for estimates of abundance from electroshocking can be ≥100% when densities are low (<10 fish/100 m^2), and do not decline to <50% until densities approach 100 fish/100 m^2 (Paller, 1995). CV for catch estimates from gillnets are ~75% (Craig *et al.*, 1986). These high variances, plus a tendency for CV on an untransformed scale or variances on a log scale to be higher at lower abundances, usually mean that many fish from many sites must be collected to provide adequate statistical power. The more abundant smaller fish or juveniles will usually provide greater power than larger adult fish.

Direct estimates of abundance are biologically relevant, and abundances will respond, usually negatively, to metal contamination and acidification (Munkittrick and Dixon, 1989b; Shuter, 1990). As a non-specific response, abundance will also be affected by other factors, especially exploitation. However, non-specificity is not necessarily a problem. Population and individual-level assessments can detect indirect effects from metals (e.g., from effects on prey), effects from increased suspended or deposited solids concentrations, effects from habitat or flow alterations from placer mining, and effects from various physical alterations from road construction associated with mine development (MacDonald *et al.*, 1991). Few if any of the sub-organism tools discussed in this report would be able to detect effects not due to metal contamination or acidification.

The real problem with direct population-level assessments is that large sample sizes in terms of numbers of fish and numbers of sites are required to provide adequate statistical power. Destructive sampling will often be socially or politically unacceptable. For these reasons, direct population-level assessments (effectively CPUE), especially for adults of large species, are not recommended as a stand-alone tool for mine environmental monitoring; indirect assessments (i.e., AFS) will usually be superior.

The AETE should avoid taking the approach adopted by the Canadian pulp and paper EEM program (EC/DFO, 1995). In that program, abundance or CPUE data are collected for adults of the same species used for AFS. Sample sizes, and especially numbers of sample sites, for the AFS are not adequate for a direct assessment of population-level effects using CPUE. Instead, the AFS approach should be used for adults, and abundance or CPUE data could be collected for *juveniles or larvae*. That would expand the assessment to most of the life cycle, and data on juvenile growth could also be easily obtained. This is effectively the approach adopted by the Swedish EPA for monitoring pulp mill impacts on fish (Sandström, 1994, 1996).

If small species are used for an indirect or AFS approach, then collection of abundance and CPUE data for adults may occasionally be possible for little additional cost. If data are robust enough for quantitative community-level assessments (Section 4.3), then those data will obviously be robust enough for population-level assessments for the most abundant species. It would also be relatively inexpensive to collect AFS-type data on subsamples of those abundant species.

There may be other opportunities in mine environmental monitoring programs to combine direct and indirect population-level assessments and possibly community-level assessments.

Those opportunities should be realized on a site-specific basis when the costs of doing so are low.

3.4.2 Indirect Measures (Adult Fish Survey)

Shuter (1990), Gibbons and Munkittrick (1994), EC/DFO (1993, 1995) Sandström (1996) and Sandström *et al.* (1997) provide recent reviews of indirect population assessments and AFS. Section 4.2 provides specific case studies and more details; Table 2 provides a summary evaluation. Basically, AFS measure reproductive effort, growth, condition and age structure (as a surrogate for mortality). Effects on those parameters can be combined in population models to provide quantitative estimates of effects on abundance (e.g., Barnthouse *et al.*, 1987, 1989, 1990; Shuter, 1990). However, interpretation is usually qualitative (e.g., as in Gibbons and Munkittrick [1994]) rather than quantitative.

Indirect population-level assessments should be sensitive to metals since the component variables are sensitive to metals (Section 3.3). The AFS approach has been used to assess direct and indirect effects from metal mines (e.g., Munkittrick and Dixon, 1988; Munkittrick *et al.*, 1991; references cited in those papers), although it has been used much more frequently to assess impacts from pulp and paper mills (see Sandström [1996] for a review).

The key advantage of AFS over direct population-level assessments (and community assessments) is that fewer fish are required. Sample sizes and statistical power for AFS are discussed in detail in FS EWG (1997); usually 20 - 30 fish per sex per species per sample area are adequate. Thus, AFS are likely to be more successful than alternatives and are recommended for that reason. However, it should be recognized that many problems remain with AFS and that the approach is unlikely to succeed at all metal mine sites. There is also no strong biological rationale for preferring AFS assessments to direct population or community assessments, so whenever the latter are possible and cost-effective they should be considered. Also, the results of the first sample year of the pulp and paper EEM indicated that the AFS approach should be expanded from that outlined in EC/DFO (1995) to include the use of small as well as large species, wild or caged bivalves, and surveys of juvenile growth and abundance where adults are rare or unavailable (FS EWG, 1997) (Section 4.2).

3.4.3 Population Genetic Structure

Other tools to determine population effects, such as genetic structure, are being explored. Aquatic organisms are affected by pollutants at levels of biological organization ranging from subcellular to community. At the population level, a strong lethal effect decreases total abundance. With less severe effects, individuals (and their offspring) resistant to contaminant toxicity may replace those suffering reduced survival or reproductive output. If there is a genetic component underlying variation in resistance, pollutants can act as selective force, altering the genetic structure of populations before eliminating them. Populations in contaminated and uncontaminated environments should differ in the frequencies of genotypes that confer differential fitness under contaminant exposure (Nevo *et al.*, 1983; Bayne, 1987).

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Electrophoretic analysis of allelic variants of enzymatic proteins (allozymes) has been an important tool for the study of population genetic structure for several decades (Richardson *et al.*, 1986). Metal contaminant-related variation in allozyme genotype frequencies has been reported in field observations of marine shrimp and gastropods (Nevo *et al.*, 1984; Nevo, 1993), barnacles (Patarnello *et al.*, 1991; Montero *et al.*, 1994), freshwater insects (Benton and Guttman, 1990) and freshwater fish (Gillespie and Guttman, 1989; Heagler *et al.*, 1993; Roark and Brown, 1996). Differential responses to metal and metalloid toxicity are related in part to genetic factors (Mulvey and Diamond, 1991; Forbes, 1996; Schlueter *et al.*, 1997). For these reasons, the analysis of allozyme genotypes or other indicators of genetic structure has been suggested as a means of detecting early disturbances to populations due to environmental pollution. However, further field testing of these methods is warranted before widespread application in monitoring programs.

3.5 COMMUNITY

Fausch *et al.* (1990) provide an excellent review and discussion of the use of fish community surveys (FCS) in environmental monitoring. Table 3 provides a summary evaluation. Community assessments can range from qualitative surveys (presence/absence; richness) to more quantitative multivariate and index-based approaches. Community-level assessments are presumably more relevant biologically than population-level assessments because they are an extension of the latter to more species and to a higher level of organization.

Although there are many examples of fish community responses to anthropogenic activities, recent FCS specifically examining the effects of metal contamination from mining activities are rare (Section 4.3). However, there are numerous published studies relating changes in fish communities, particularly in lakes, to acidification (e.g., Rago and Wiener, 1986; Jackson and Harvey, 1989). Regional assessments relating fish communities to water quality parameters often include metals, although not necessarily from mines (e.g., Leonard and Orth, 1986; Van Hassel *et al.*, 1988). Fish communities are presumably sensitive to metal contamination, and to increases in suspended or deposited solids and habitat or flow alteration, since many individual species are. The direction of effects may differ among species, particularly if sensitive species are replaced by more tolerant competitors.

The key problem with FCS is the same as that with direct population assessment - large sample sizes in terms of numbers of fish and sample sites are required. More effort is usually required to capture and count or weigh more species. Therefore, FCS are not recommended for general inclusion in environmental monitoring programs at metal mines. However, at specific sites, community assessments may be practical and cost-effective. FCS are recommended for regional monitoring of environmental quality, although a nationwide monitoring program may not be the best vehicle for application of FCS at a regional level. Communities of small fish in streams or littoral areas of lakes have been used most frequently in other assessments, and would be most suitable for national or regional level programs.

4.0
DISCUSSION

4.1 USE OF CHEMICAL AND BIOCHEMICAL TOOLS FOR AQUATIC EFFECTS MONITORING

There are several properties of chemical and biochemical methods that warrant their inclusion in aquatic effects monitoring programs.

- In assessing biological responses to environmental disturbances within the framework of the hierarchy of biological organization (Figure 1), responses to environmental disturbances at the chemical- and biochemical-levels of biological organization are expected to indicate impairment before assessments at higher levels (i.e., individual, population and community). As tools in an environmental effects monitoring program, bioaccumulation (metals in tissues) and biomarker (biochemical assessments) measurements are thus potential "early warnings" of effects at the more ecologically-relevant higher levels.

- Some bioaccumulation and biomarker responses are also more indicative of specific disturbances than higher level responses. For example, metal-enriched mine effluents could result in: (a) elevated concentrations of metals in gill tissues; and, (b) reduced abundance of fish. Whereas high levels of metals in tissues must have come from environmental metal contamination, reduced population abundance could have alternate causes.

- Impairment at low levels of biological organization are more reversible than impairment at higher levels. Detection of bioaccumulation and biomarker responses thus allows more opportunity for mitigation of disturbances than does the determination of damage to populations and communities.

Recommendation of specific methods for inclusion in aquatic effects monitoring programs for the mining industry depends on their: (a) relations to more ecologically relevant effects; (b) responsiveness to disturbances from mining activities; and, (c) ease of application. In the following subsections, the suitability of bioaccumulation and biomarker methods for monitoring programs at metal mines based on the above criteria are discussed. The final subsection deals with their integration into fish surveys.

4.1.1 Metals in Tissues

Depending on the degree to which metal regulation occurs, observations of metal bioaccumulation in fish tissue can potentially: (a) indicate exposure to contaminants; and, (b) predict biochemical and tissue-level responses to metal stress. Studies to date provide more support for the former rather than the latter use. Concentrations of metals in fish vary widely, depending on metal, degree of exposure, fish species and tissue type. Best relationships between tissue and environmental levels have been determined using bone, gill, liver and kidney. Bone concentrations are expected to be the most indicative of long-term metal exposure; blood concentrations are indicative of short-term exposure. Relationships between elevated tissue metal levels and degree of metal toxicity are not well established. Concentrations of metals in tissue are only a part of the "larger picture" of metal metabolism that includes uptake, regulation and detoxification. Establishing dose-response relationships between environmental, tissue metal concentrations, and metal toxicity is likely to be difficult without consideration of these additional processes (including the role of metallothioneins, which is the subject of another review).

For assessments of effects of mining activities on fish, tissue metal concentrations are among the few means of determining exposure to metals. In case studies of aquatic effects of metal mines in Canada reviewed by AQUAMIN (1996), elevated metal concentrations in fish tissue were reported in 6 (out of 18) studies. These results were considered indicative of exposure of fish to mining effluent. Monitoring programs should include analyses of tissues for metal concentrations as part of a series of assessment methods. These should be standardized according to tissue type, fish species, and fish size or age. Collection and analytical methods are relatively easy and inexpensive compared to other assessment tools.

4.1.2 Biomarkers

Ion Status — Plasma ion levels can be indicative of iono- and osmoregulatory stress related to acutely acidic and metal contaminated conditions. In particular, Na loss has been the most extensively validated in the field environment. Folmar (1993) tabulates normal or "reference" values for various plasma parameters and expected effects of selected contaminants. Blood ion status variables are not well correlated with population-level effects, and are therefore of limited value for use alone as biomarkers.

Inhibitory/Inducible Enzymes and Other Proteins — Evidence to date suggests that few enzymes or other inducible proteins are suitable biomarkers for metal contamination. ALAD activity (inhibited by Pb) and gill ATPase activity (generally inhibited by metals) are potentially useful with whole organism or higher level effects. Further studies are required to link enzyme activities with fish health before these methods can serve as early indicators of metal-induced stress. Responses are strongly affected by the fish's physiological state and confounded by environmental xenobiotic compounds.

Lipids and Carbohydrates — Lipid and carbohydrate (glycogen, glucose) concentrations are rough indicators of the energy status of organisms, and thus potentially predictive of energy-consuming processes such as growth and reproduction. Analyses are simple, but variation due to additional factors can be great. Use as biomarkers of chronic metal stress (with other indicators) could be worthwhile.

Biochemical Indicators of Growth — Scope for growth is the most suitable among biochemical indicators of growth for application in environmental monitoring at metal mines. Though not as well studied in fish, it has been extensively field tested with marine mussels. Most of these indicators were developed to detect minor changes in growth rate and short-term exposures (Munkittrick and Power, 1990). All biochemical indicators of growth are influenced by the physiological state of the organism, and other modifying environmental factors. Consequently, they could be too sensitive and variable for long-term field conditions.

Biochemical Indicators of Reproduction — There are currently no reliably field-tested biochemical methods that are indicative of reproductive responses of fish to metal pollution.

Biochemical Indicators of Immunity — Immunological biomarkers are not currently suitable for assessing metal contamination in field conditions.

Biochemical Indicators of Respiration — Environmental concentrations of metals are not usually high enough to induce respiratory responses in fish. Furthermore, because respiratory distress is an acute toxicity response, it cannot be considered as an early indicator of mining-related impacts and would not be useful in a monitoring program.

Genotoxic Responses — Metals are not strong carcinogens or mutagens. None of the available studies recommended the use of genotoxic responses in fish to monitor effects of metal contaminants.

4.1.3 Relation to Individual- and Population-Level Assessments

The use of chemical and biochemical methods involving fish in aquatic effects monitoring programs for pulp and paper mill effluents has been evaluated by independent groups of experts in Canada (FS EWG, 1997) and Sweden (Sandström *et al.*, 1997). Both of these groups cited the importance of: (a) performing tissue contaminant and biomarker measurements in concert with assessments of higher order effects on whole organisms and populations; and, (b) linking the biomarker responses that are selected for observation to ecologically relevant attributes of fish populations. Monitoring for toxicological effects should involve suitable sentinel species, because observed effects are meaningful only if sufficiently stationary populations exist in the receiving waters. Exposure of these populations to the effluents of interest needs to be demonstrated. To this end, there is a role for bioaccumulation and biomarker observations.

4.1.4 Recommendations

The objectives of an aquatic EEM program for mining activities were suggested by AQUAMIN (1996) to involve two phases:

1. Site characterization
2. Field investigation and monitoring

The environmental components would include water, sediment, benthic biota, and fishes. Depending on the outcome of the site characterization, the second phase could involve all or some of the following:

- Assessment of current conditions
- Focussed monitoring
- Investigation of potential cause(s)
- Periodic monitoring

Based on the review of chemical, biomarker and tissue-level methods for assessing effects on fishes, the measurement of metal concentrations in tissues is the only procedure that appears warranted for inclusion in a *routine* EEM program. Other methods reviewed are currently of limited use due to:

- Lack of relevance as either indicators of exposure to metal mine effluent or predictors of higher-level (individual and above) effects.
- Non-specificity to metals or low pH.
- Potentially high temporal variability.
- Difficulty in application because of sampling requirements and availability of trained analysts.

In investigative studies, however, certain techniques, such as specific inhibitory or inducible enzymes and tissue pathology, could prove useful in linking observed higher-level damage in fish to mining-related contamination. Such studies would likely be conducted only in certain situations and involve a more extensive sampling program than that designed for a yearly or multi-year-cycle EEM-type program.

4.2 APPLICATION OF ADULT FISH SURVEYS (AFS)

This section provides a detailed review and evaluation of the use and suitability of adult fish surveys (=indirect population assessment) for potential application at metal mines. Section 3.3 provides a detailed review and evaluation of component variables of the AFS (growth, reproduction and condition); Section 3.4.2 provides a brief summary and evaluation of AFS (see also Table 2).

The review and evaluation provided in the following subsections rely extensively on experiences with AFS in monitoring pulp and paper mills in Canada (FS EWG, 1997) and Sweden (Sandström *et al.*, 1997). Although most Swedish mills are coastal, discharging to the Gulf of Bothnia, the Gulf has a low salinity and the fish monitored are freshwater species (Sandström, 1994, 1996).

4.2.1 Background and Rationale

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Harris (1995) indicates that fish possess three main attributes which make them useful for environmental monitoring programs:

- Fish are sensitive to most forms of human disturbance (this can be a disadvantage in assessments of specific disturbances).
- Fish are useful for monitoring at all levels of biological organization.
- Fish monitoring programs have a favourable benefit-cost ratio (a debatable point in marine and estuarine environments).

Key questions in determining how to apply fish monitoring are:

- Why conduct higher-level (population or community) assessments?
- Why conduct an AFS as opposed to other higher-level alternatives?

The answer to the first question is that higher-level assessments are more relevant than lower-level assessments (Figure 2); the answer to the second question is that an AFS will be the most practical higher-level tool at most mine sites.

Ideally, to assess the effects of anthropogenic activities on fish populations, fisheries biologists would use accurate and precise estimates of abundance (or biomass). In practice, these estimates are difficult or impossible to obtain at a reasonable cost (Section 3.4.1). Therefore, fisheries biologists often use other variables, such as those measured in an AFS, to assess the status of fish populations and predict future changes in abundance (Ricker, 1975; Getz and Haight, 1989; Smith, 1994). Abundance and biomass, and their changes over time, depend on (Barnthouse *et al.*, 1987; Getz and Haight, 1989; Shuter, 1990):

- Age- or stage-specific survival (or its inverse, mortality).
- Growth.
- Fecundity.

Growth and fecundity are measured directly in AFS; age structure acts as a qualitative surrogate for survival (Shuter, 1990; Gibbons and Munkittrick, 1994; Section 4.2.2.5). Age at maturity can also be an important variable in species with indeterminate growth, such as fish. Although it is possible to use AFS results in simple population models to provide quantitative estimates of population-level effects, it would be safer to interpret results qualitatively (i.e., as in Shuter [1990], or Gibbons and Munkittrick [1994]). Despite the limitations of AFS variables, extrapolations of population effects from lower-level (i.e., sub-organism) effects or from laboratory toxicity tests are probably more uncertain (e.g., Barnthouse *et al.*, 1990).

4.2.2 Study Design and Methods

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EC/DFO (1995) provides the most recent complete description of study design and methods for AFS. Shuter (1990) and Sandström (1996) provide general descriptions of study designs and methods; Munkittrick (1992) reviews study design considerations.

4.2.2.1 Overall Strategy

For an AFS to be successful:

- The target fish species must be available and abundant enough to provide adequate sample sizes (= availability).
- Exposure to metals and/or to increased suspended or deposited solids, habitat or flow alteration, and any other disturbances caused by mining must differ among sample sites (i.e., between exposed and reference areas [= exposure]).
- The necessary biological parameters (i.e., age, growth, reproduction) must be measurable on the fish with a reasonable degree of precision and accuracy (= measurement).

The sequence availability→ exposure→ measurement can be used to design individual AFS programs, evaluate case studies, and identify weak links in the overall approach.

Because population-level tools are non-specific, responding to many factors other than mining activity, a key element in design is to eliminate or reduce the effects of those factors. Confounding effects are removed or reduced by careful selection of study species, sample sites, and variables. If this is done, then non-specificity becomes an advantage, allowing indirect effects, and effects from factors other than metal contamination, to be detected.

4.2.2.2 Species Selection

Species used in AFS should be abundant and easily captured (i.e., available) in both exposed and reference areas, sensitive to mining activities, sedentary enough to be exposed to those activities for months or years rather than weeks, and suitable for measuring AFS variables. In general, large mobile species, and commercially or recreationally exploited species, will not be suitable for AFS. If "fish"is considered to include shellfish, then crustaceans will not be suitable because they cannot easily be aged. Beyond that, species selection will depend on characteristics of the study site and the biology of potential candidate species.

Previous authors (e.g., Munkittrick, 1992; EC/DFO, 1995) have provided fairly restrictive criteria for sentinel species selection. The criteria generally match characteristics of intermediate-size benthivores such as suckers. Since suckers have been successfully used in many AFS studies (Munkittrick and Dixon, 1989b; Sandström, 1996), intermediate-size benthivores will be suitable at many mine sites. However, smaller fish species (e.g., darters, minnows, sculpins), and bivalve shellfish, should also be considered as potential sentinel species (Gibbons *et al.*, 1998a,b). Shuter (1990) noted that small species were more sensitive to acidification than larger species, or at least responded more quickly to changes in pH. On a practical level, small species are usually more abundant, easier to capture, and more sedentary than larger species. In freshwater fish, home range size is positively correlated with body size (Minns, 1995). Variables such as age, age at maturity, and fecundity are often easier to measure precisely and accurately than on larger fish.

There can be disadvantages to using small fish in AFS. Often little is known about their basic biology, and particularly their spawning habits, making it difficult to determine the best sample areas, times and methods. Some species are multiple spawners, producing more than one clutch of mature ova every year (e.g., Heins and Rabito, 1986; Burt *et al.*, 1988; Paine, 1990). Reproductive effort in these species is impossible to estimate from a single sample, because the reproductive tissue can be turned over almost completely between clutches (i.e., most of the mass of ova in the ovary will be spawned, and then a new clutch of mature ova will be developed). The number of clutches produced during the spawning season becomes an important reproductive variable and is almost impossible to estimate for an individual female in the field, even with frequent sampling.

For example, females of the minnow *Notropis leedsi* can produce ≥10 clutches of ~100 mature ova in a single spawning season (Heins and Rabito, 1986). Counts of mature ova, and ovary weights, at any single time, even immediately before spawning, seriously underestimate reproductive effort for that species and other multiple spawners. Variances of fecundity estimates and ovary weights will also be high because females with ova and clutches at various stages of maturity may be present at any sample time.

There is empirical evidence that the frequency and extent of multiple spawning decreases with increasing latitude (Hubbs, 1958; Paine, 1990), so multiple spawning may not be a problem at many Canadian mine sites. *Notropis leedsi*, discussed in the preceding paragraph, occurs in the southeastern U.S., and spawns from May through September. Spawning seasons for Canadian species of *Notropis* are shorter, although spawning may still extend over more than one month for some species (Scott and Crossman, 1973). Multiple or protracted spawning may also be limited in species in which the male guards the developing embryos, and in other species with large ova (Paine, 1990; Gibbons *et al.*, 1998a). As a general rule, multiple spawning will be less likely to occur in species with short spawning seasons, and those species are preferred for AFS.

The length of the spawning season for most species is rarely known for specific sites, or even for Canadian north temperate and sub-arctic regions. Since most small species are spring spawners, the length of the spawning season can be estimated directly by preliminary sampling during spring and summer. Alternatively, the presence of poorly differentiated ova and spent females in late summer or fall samples is usually evidence of multiple or protracted spawning. Two or more distinct size classes or maturity stages of ova will usually be present in multiple spawners collected in spring prior to spawning (e.g., Heinz and Rabito, 1986).

Results from the first cycle of the pulp and paper EEM clearly indicated that AFS using larger fish are unlikely to be successful in marine and estuarine environments (FS EWG, 1997). To date, the AETE evaluation has been restricted to freshwater environments. For the purposes of this report, the AETE need only be aware that alternatives to large finfish such as bivalves or smaller finfish will be required for mines in marine and estuarine environments. The FS

EWG report from the pulp and paper EEM contains a detailed evaluation of those alternatives. Also, freshwater bivalves have been successfully used for population-level and other assessments of metal contamination (Elder and Collins, 1991; MacMahon, 1991). Probably the greatest advantage of bivalves relative to finfish is that they can be caged or confined for extended periods to provide more control over exposure; the greatest disadvantage would be that reproductive effort is difficult to measure.

4.2.2.3 Sample Site Selection

There are two levels of sample site selection. First, one must define the overall spatial boundaries of the survey, and specifically the extent of exposure, and select one or more habitats to sample. In general, streams, small rivers and littoral zones of lakes are easier and less costly to sample than larger rivers and pelagic or profundal zones of lakes. Dilution will also be lower, and fish communities richer, in the former habitats than the latter. Many of the pulp mills in Canada are located on larger rivers or lakes, which reduces the pool of potential species and habitats for AFS. Mine sites are often located near smaller water bodies, which should remove some of the problems experienced in the pulp and paper EEM.

In EEM programs, the extent and location of the exposure zone are usually fixed by factors such as effluent discharge and dilution, and/or by the location of other activities such as tailings disposal and flow alterations. There is some flexibility in terms of which habitats within that zone can be sampled. However, selection of an appropriate reference area or areas is probably the most critical aspect of study design. Munkittrick (1992) and EC/DFO (1995) review reference area selection for AFS. Basically, reference areas should be as similar as possible to the exposed area, except in exposure to mining activities. In general, reference areas near the exposure area will be more similar than those further away, but the distance between sample areas should be greater for larger and more mobile fish. In rivers, upstream reference areas (i.e., internal references) are preferred, although there are downstream gradients in fish population characteristics over long distances in rivers (e.g., Munkittrick, 1992; Gagnon *et al.*, 1995).

Adequate reference areas may not be available at some Canadian mine sites. For example, there may be dams and reservoirs immediately upstream of mines on rivers. In those cases, a far-field area could be used as a reference, or a number of areas representing an exposure gradient could be sampled. This approach would require either well-separated areas for larger species, or the use of small species. Any AFS study will be improved if multiple references are sampled, although the costs of doing so can be high for large mobile fish.

4.2.2.4 Sampling Methods

Section 3.4.1 reviews and discusses sampling methods, providing general references. One major problem with AFS conducted in the pulp and paper EEM program was that too few fish (i.e., <20 fish per species per sex per sample area) were captured in many studies (FS EWG, 1997). The factors responsible for the limited capture success were numerous. Also, capture success was generally greater in freshwater environments than in marine or estuarine environments. The AETE can also anticipate greater capture success in the metal mining context because the water bodies and fish sampled will probably be smaller than in the pulp and paper EEM. However, consultants conducting preliminary studies for the AETE also experienced difficulties collecting sufficient fish at some Canadian mine sites (EVS, ESP and JWEL, 1996). The limited capture success was the major reason why direct population-level assessments, and community-level assessments, are not recommended for general use in environmental monitoring at metal mines.

There are several ways to improve capture success in AFS, although there will undoubtedly be sites where sufficient numbers of fish cannot be collected at a reasonable cost. First, and most obvious, target or sentinel species should be abundant, especially in the exposure zone. That is the major reason why we recommend expanding the pool of potential species to include small finfish and bivalves. Second, sampling methods should be targeted towards *adults* of the specific species chosen; gear will often have to be modified. For example, fixed- and not variable-mesh gillnets should be used to capture only the size range of interest (Munkittrick, 1992). Third, reasonable effort must be expended to collect the fish, and more than one type of capture method or gear may be required. For example, 100 m of variable-mesh gillnet set for one or two lifts may not be adequate effort or gear; 1000 m of fixed-mesh gillnet set for as many or more lifts may be more desirable, depending on the water body.

4.2.2.5 AFS Variables

The basic variables measured in AFS are age, size (length and weight), and gonad weight (and fecundity in females). Growth can be calculated from size and age data; condition can be calculated from weight and length; gonad weight and fecundity are usually adjusted for body size. Section 3.3 provides more details on these measures. Age at maturity is usually mentioned as another AFS variable (e.g., Munkittrick and Dixon, 1989b; Gibbons and Munkittrick, 1994) but can be difficult to estimate and analyze without large sample sizes (e.g., EC/DFO, 1995). Liver weight is also usually included in AFS.

In AFS, age structure is used as a surrogate for estimating effects on survival. Basically, the life stage(s) most affected are inferred from the age distributions, although that can be difficult if only adults are sampled (Shuter, 1990; Gibbons and Munkittrick, 1994). Age-specific survival or mortality could be estimated from time series of abundances of single age classes or cohorts, although that requires good estimates of absolute or relative abundances. An alternative is to estimate survival from age distributions in a single sample or several samples, a method known as catch curve analysis (Ricker, 1975). Johnson and Landahl (1994) provide an example of the application of catch curve analysis to assessment of contaminant effects on survival of English sole (*Pleuronectes vetulus*). Theoretically, catch curve analysis could also be applied to AFS age distributions to estimate survival or mortality rates. In practice, sample sizes from the AFS would be too small to obtain reliable survival estimates from catch curve analysis. For example, the catch curve analysis in Johnson and Landahl (1994) was based on samples of fish from four years, with >100 fish caught in each year (total catch = 1188). Therefore, age structure in the AFS should be treated as a qualitative surrogate, and not a direct quantitative measure, of potential effects on survival.

Supplementary variables in AFS could include lower-level measures on fish discussed in Sections 3.1 and 3.2, water quality, and habitat variables (depth, velocity, substrate, etc.). If metal contamination exists then either tissue metal or metallothionein concentrations should be measured as an internal indicator of exposure (i.e., "tracer" in the lexicon of the pulp and paper EEM program). Exposure is more difficult to assess, other than by residency (i.e., presence in the exposure zone = exposure, with no consideration for duration), when increased suspended or deposited solids, or flow or habitat alteration, are major concerns. In those cases, measures of suspended or deposited solids and habitat variables become exposure indicators, although external to the fish.

4.2.3 Programs and Case Studies

4.2.3.1 Programs

An AFS is an important part of mandated monitoring programs for Canadian and Swedish pulp and paper mills (e.g., EC/DFO, 1995; Sandström *et al.*, 1997). The Swedish program also includes measurements of sub-organism (mostly biochemical) and community parameters. Australia has monitoring requirements for new bleached eucalypt Kraft mills (Keough and Mapstone, 1995). All of their mills discharge to marine environments, and if population-level assessments are used, it appears likely they will focus on plants rather than fish. Similarly, snails rather than fish were recommended for population-level monitoring in the Alligator Rivers Region (ARR), the site of extensive mining (mostly for uranium) in Australia (Humphrey *et al.*, 1995).

We are not aware of any country which requires an adult fish survey or similar population-level assessment in a mandated national program for metal mines. Fish community surveys are often included, although not legislated, in U.S. National Pollutant Discharge Elimination System (NPDES) Programs. Although Australia may be receptive to the use of fish for monitoring environmental impacts and water quality, recommended tools were behaviourial responses, direct population assessments over broad spatial scales using fisheries data, and community surveys (Harris, 1995).

4.2.3.2 Case Studies

Case studies reviewed as part of our evaluation were drawn from the current primary literature as grey literature was difficult to access. Our search included all issues of *Environmental, Toxicology and Chemistry* (*ET&C*) and *Canadian Journal of Fisheries and Aquatic Sciences* (*CJFAS*) from 1988-96 since these two journals are the most likely to publish relevant case studies. For example, we recently published an article in *ET&C* which included an AFS-type assessment of PAH impacts on crabs (Paine *et al.*, 1996). The results of the literature search were as follows:

- Only two examples of the use of an AFS-type approach to monitor impacts from mines, or even from other sources of metals (e.g., smelters) were identified. One of these case studies (Munkittrick *et al.*, 1991) was simply a follow-up of an earlier study (Munkittrick and Dixon, 1988), previously cited in Munkittrick and

Power (1990). The other study stretched the definitions of both "relevant" and "AFS-type".

- Numerous examples of AFS and other population-level studies of impacts from pulp and paper mills (reviewed in Sandström, 1996) and some other industrial activities (e.g., power plants) were identified. Population-level studies of the effects of acidification were also common in the 1980s (reviewed in Munkittrick and Dixon, 1989b; Shuter, 1990).

- Many field and laboratory studies of the effects of metals (not necessarily from mines) on sub-organism parameters were identified. Few if any of these studies reported effects on condition, growth, reproduction, and/or survival even though it was clear that the necessary data were available (especially in laboratory studies) or could have been obtained at little extra cost (i.e., the fish were already caught for other purposes). The best example of this general failure to address population-level effects is a study conducted at the Clark Forks site, Montana, which is heavily contaminated by metals from nearby mines (*ET&C*, Vol. 13(12); several papers are included in this issue). The study included almost every monitoring tool but measurement of AFS variables and abundances, even though effects on fish (primarily rainbow trout) were a major concern. Sub-organism indicators were measured on fish captured in the field. In fairness, AFS-type and other fisheries data are available in some state agency reports cited in Pascoe *et al.* (1994), but it is puzzling that more of those data were not included in the special issue of *ET&C.*

In conclusion, our literature search was able to uncover examples where the AFS approach was applied, but the approach is rarely applied to assess the impacts of mines and/or metals even when it could be for little or no cost. The investigators conducting the studies were not averse to field work, potentially non-specific tools, or higher-level assessments; macroinvertebrate community surveys were included in many studies. There appears to be a clear division between environmental toxicologists and fisheries biologists in metal mine monitoring that is less evident in monitoring programs conducted for other industries.

The most relevant case history (Munkittrick *et al.*, 1991) indicated that metal mine effects on age, fecundity and growth of white suckers described in Munkittrick and Dixon (1988) were largely the result of reduced prey availability (i.e., metal effects on macroinvertebrates). There were also some direct metal effects on sucker larvae. Carline and Jobsis (1993) examined the effects of metals deposited from aerial emissions from a Pennsylvania smelter on abundance and growth of trout (brook and brown) and white suckers. There was no relationship between juvenile growth of any species and distance from the smelter. Those results were hardly surprising, as tissue metals were not markedly elevated, and the smelter had not been operating for six years.

We also briefly reviewed the AQUAMIN (1996) Final Report, and associated supporting documents. The supporting documents review and summarize monitoring programs from Canadian mine sites, usually published in consultants' reports (i.e., the grey literature). Metal residues in fish tissue were commonly measured, usually to compare concentrations from reference versus exposed areas. The reviewers occasionally noted that life history variables such as age, length and weight were also measured on the same fish. However, the reviewers never indicated whether there was any association between tissue contamination and effects on life history variables, or even if those variables differed between reference and exposed fish. As in the survey of the primary literature, either the AQUAMIN reviewers or the original report authors ignored opportunities to link tissue contamination with more relevant higher-level demographic effects. In fairness, sample sizes were limited in many of the studies, and the major concern was often contamination of edible fish, rather than effects on the fish themselves.

Some Canadian mine monitoring programs reviewed by AQUAMIN included direct assessments of effects on populations, estimated from comparisons of CPUE among sites or times. Life history or AFS variables were also measured in some of these studies. However, there was no program in which an indirect or AFS approach was the primary tool used to assess population-level effects. Considering the limited numbers of fish captured in many of the direct population surveys, an AFS approach would probably have been superior.

4.2.4 Evaluation

The AETE screened monitoring tools based on two criteria:

- Has the method been successfully applied in the field?
- Is the method cost-effective?

The answer to the first question is obviously "Yes, although rarely to mines/metals" (Section 4.2.3). The AETE will have more opportunity to evaluate the feasibility of AFS based on results of 1997 field studies. Many problems can be solved following guidance provided in Section 4.2.2, and by planning and evaluating each study following the availability → exposure → measurement sequence. The pulp and paper FS EWG report (FS EWG, 1997) also has additional practical guidance. Nevertheless, the AFS will not be suitable at all mine sites, despite best efforts, and the AETE should consider some of the alternatives discussed in the FS EWG report (FS EWG, 1997).

Costs of an AFS are difficult to evaluate, although some are provided in EVS, ESP and JWEL (1997). If a monitoring program consists only of an AFS, then the costs of field trips and sample collection (i.e., major costs) must be assigned entirely to the AFS. However, if other field work (e.g., water and sediment quality monitoring; macroinvertebrate surveys) is conducted at the same time, then the costs of field trips can be shared between the AFS and other components of the monitoring program. If fish are collected for other purposes (e.g., metallothionein or tissue metal levels; histopathology), then measurements of most or all of the AFS variables on those fish should certainly be made; the costs of the AFS measurements are relatively low (Section 3.3). Assuming that some sort of fish survey is conducted, the important issue will be to decide the appropriate allocation of cost and effort among AFS variables, tracers or exposure indicators, and sub-organism indicators (the order given is our assessment of priorities). Our experience with the AFS in the pulp and paper EEM program is that costs are ~$20 - 50K, exclusive of data analysis and report writing, comparable to those for macroinvertebrate surveys or effluent toxicity testing. These costs apply to freshwater environments, with target species and sampling methods known from previous studies. Costs will escalate if tracers (metals, metallothioneins) are measured on individual fish.

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4.2.5 Recommendations

An AFS is recommended for general use in monitoring at metal mines largely by default - it is the most practical population- or higher-level tool currently available. The case studies in Section 4.2.3.2 indicated that the necessary data can often be obtained from surveys of tissue contamination and lower-level effects. Therefore, AFS or life history variables should be measured and analyzed if any fish survey is conducted for metal mine EEM. Based on the experience of the pulp and paper EEM, and previous AETE field work, the AFS will not be successful at every site. Therefore, the AETE should consider alternatives at sites where the AFS is demonstrably unsuitable, and encourage site-specific flexibility in monitoring higher-level effects on fish.

4.3 APPLICATION OF FISH COMMUNITY SURVEYS (FCS)

This section provides a detailed review and evaluation of the use and suitability of fish community survey (FCS) for monitoring at metal mines. Section 3.5 provides a brief summary of FCS (see also Table 3).

Fausch *et al.* (1990) provide a good review and evaluation of the use of FCS in environmental monitoring; Karr *et al.* (1986) provide the original rationale, development, protocol, and examples of the Index of Biotic Integrity (IBI) approach; Klemm *et al.* (1990) provide the U.S. EPA protocol for rapid assessment of IBI, which is not radically different from that in Karr *et al.* (1986). Matthews and Heins (1987) provide a general review of community ecology in North American stream fish, which includes case studies. Hocutt and Stauffer (1980), although outdated, provides a good review and case studies of the application of fish biomonitoring, including FCS. MacDonald *et al.* (1991) review methods for FCS, and briefly discuss the usefulness of community measures for assessing effects from mining and road construction.

4.3.1 Background and Rationale

The advantages of using fish in biomonitoring are discussed in Section 4.2.1. Fausch *et al.* (1990) emphasize the integrative nature and societal relevance of FCS. They consider non-specificity (i.e., the ability of fish communities to respond to many different stresses) as a positive, as does Harris (1995). FCS are not just monitoring tools; the IBI and related indices have been incorporated into regulatory and management programs, especially in the U.S. (Section 4.3.3). Therefore, FCS are politically as well as biologically relevant.

4.3.1.1 FCS Approaches

Fausch *et al.* (1990) identify four approaches to FCS:

- Indicator taxa/guilds
- Simple indices (i.e., richness, diversity, evenness, etc.)

- Multivariate analyses
- The IBI and related summary indices

Surveys of indicator taxa or guilds focus on specific, usually rare or sensitive, taxa or guilds. Guilds could be based on feeding habits, habitat or reproduction. On a regional basis, these surveys are often qualitative (i.e., based on presence/absence and not abundance or CPUE). The other three approaches are defined mostly on how one treats the data, although there may also be an increase in effort/cost from simple indices ➜ multivariate analyses ➜ IBI. There can also be differences in appropriate scales among approaches, which are important for the design of a metal mine monitoring program.

4.3.2 Study Design and Methods

The overall strategy in designing and evaluating FCS differs somewhat from that for an AFS (Section 4.2.2.1). In both cases, fish must be available; this is even more important in an FCS because many, rather than one, fish species are targeted. In both types of survey, investigators should be confident that exposure differs among the sites sampled. Internal exposure indicators (e.g., tracers such as tissue metals) are rarely measured in FCS. The presence of a fish in an exposure zone is considered adequate evidence of exposure, although exposure indicators external to the fish (i.e., water or sediment chemistry) are usually measured. Considering that the species used in an AFS could easily be members of the communities sampled in FCS, the different emphasis on measures of exposure is puzzling. Finally, measurement (i.e., of variables) is of lesser importance for FCS than AFS since almost any fish can be identified and counted.

4.3.2.1 Study Design

Study design in an FCS involves selecting the appropriate communities, sites and times. FCS are usually conducted on communities of small fish in streams or littoral zones of lakes, to restrict the effort required to sample adequate numbers of fish. Regional FCS may be based on larger fish, in rivers or lakes, especially if data are already available from other sources.

Karr *et al.* (1986) discuss the selection of sample sites and times in detail. In an FCS, replicates are spatially separated segments and not individual fish within exposed and reference areas. These segments are usually 50-100 m long in streams, although they may be 0.5-1.0 km long in rivers (Karr *et al.*, 1986). If blocking nets are used, the segments need not be widely separated from each other. Paller (1995) used 100-m segments separated by only 10 m, which probably would introduce some risk that sampling one segment would disturb the fish in adjacent segments. In surveys of lake littoral areas, segments should be well separated (i.e., separate bays or coves) unless blocking nets are used. Ideally, replicate segments should be randomly selected from all possible segments within an area. In stream surveys, where there may be strong exposure gradients and small areas of homogeneous exposure, sampling may have to be complete, with all segments within an area sampled.

Sample sizes should be at least 5 segments per area, and 10-20 would be preferable (EVS, ESP and JWEL, 1997). Most single-stream studies cited in this report used ≥5 segments per area and often 5-10 areas. Realistically, multiple reference areas, and possibly multiple exposed areas, should be considered a "must" for development of IBI (Section 3.4.2). Another alternative, probably more suitable for small-scale point-source impact assessments, is to use different sample times (years, or months within spring-fall) as replicate blocks, with only one sample per area taken at each time. This approach would be costly at remote mine sites, unless on-site mine personnel rather than consultants conducted the sampling. Regardless of the approach adopted, sampling effort and spatial or temporal scale are much greater than in an AFS. Regional FCS often sample >100 sites, which may be reaches, streams, or individual lakes.

A further consideration is the number of fish to be collected within each sample segment. CV for untransformed, or variances for log-transformed, abundances will decrease with increasing numbers of fish collected at lower abundances (Paller, 1995; Green [1989] provides the theoretical background). Richness will also be correlated with the number of fish collected, up to some asymptote. Most of the studies cited in this report collected >50 and usually >100 fish per segment, although numbers were of course lower in severely impacted sites. In a metal mine monitoring program, the optimal number of fish to be collected per segment may be lower than in U.S. studies since fewer species will be present. In any FCS, investigators need to consider whether it is better to devote more effort to collecting more fish within each segment or to sample more segments with a reduced effort within each (Jones and Stockwell, 1995; Paller, 1995).

Appropriate sample times vary among studies (Karr *et al.*, 1986). Most capture methods are more efficient at lower flows, so periods of high discharge should be avoided. Sampling in early summer will generally avoid problems created by the collection of large numbers of young-of-the-year (YOY) (i.e., restrict the survey to older juveniles and adults).

4.3.2.2 Sampling Methods

Sections 3.4.1 and 4.2.2.4 review sampling methods. In a more comprehensive or quantitative FCS, several different sampling methods or gear may be required to obtain a representative sample of the community (Karr *et al.*, 1986). In stream surveys, segments are usually blocked off with nets, then one or more electroshocking passes are made. Electroshocking will undersample some species, although it may be safe to assume that the bias is constant among sites. Lyons (1986) provides a method for using beach seines, in conjunction with blocking nets, to sample lake littoral areas. Regardless of the methods used, investigators at more northern mine sites may experience difficulties capturing sufficient numbers of fish per segment.

4.3.2.3 Variables

In the most basic FCS, fish need only be identified and counted. Usually, little additional effort is required to measure weights and lengths on all fish or on subsamples of the more abundant fish. The size data are useful for subdividing species into age or size classes for statistical analysis. For example, in streams or rivers dominated by Pacific salmon, the fish should be divided into YOY and age 1+, 2+, etc. Development of an IBI requires a brief examination of each fish for external abnormalities, which is something which could be done at little cost in most studies. One advantage of an FCS relative to an AFS is that destructive sampling is not required; the fish can be released unharmed after processing. Selected specimens of each species should be sacrificed and preserved to verify identifications.

As indicated above, tracers are rarely measured in fish in an FCS, probably because it would be too expensive to do so on several species from each segment. Tracers are measured on dominants or selected larger species in some studies (e.g., Carline and Jobsis, 1993). Water and/or sediment quality variables should be measured as external exposure indicators. As in an AFS, suspended or deposited solids levels, or habitat variables, can also serve as external exposure indicators if solids discharges or habitat alteration are concerns.

4.3.3 Programs and Case Studies

4.3.3.1 Programs

FCS are not part of any mandated monitoring program in Canada, as far as we are aware. Quebec is developing an IBI-type approach for broad-based environmental quality assessment. Ontario has had a long history of fish community studies, particularly of lake communities subject to acidification. The Ontario Ministry of Natural Resources (OMNR) and Dr. Harold Harvey of the University of Toronto maintain large databases on Ontario lakes, which include fish community data. These databases are used for regional environmental quality assessments. In the U.S. many states use an IBI approach for regional environmental quality assessment. IBI approaches are also included in NPDES monitoring for point source discharges. Harris (1995) recommended that IBI be developed in Australia, but the approach was apparently not recommended for monitoring coastal pulp mills (Keough and Mapstone, 1995) or uranium mining activity (Humphrey *et al.*, 1995). Littoral FCS are required in the Swedish EPA monitoring program for pulp and paper mills (Sandström, 1996, Sandström *et al.*, 1997) although the surveys appear to be mostly qualitative (e.g., Landner *et al.*, 1994).

We are unaware of any national, provincial or regional requirements for an FCS at mine sites in Canada. FCS may be required at individual sites, although we are again unaware of any examples. Community surveys are often included in baseline monitoring for new projects, although the surveys are largely qualitative and may only be used to select sentinel species. In the U.S., NPDES requirements for an FCS would apply to individual mines.

4.3.3.2 Case Studies

With the exception of the NPDES and the Swedish EPA requirements, FCS are generally used regionally, rather than for single point sources. Therefore, it was not surprising that we did not find any examples in the primary literature of an FCS applied at a single mine site. Presumably, results from FCS in single-site NPDES studies would be published in the grey literature (i.e., consultant reports) which would be unavailable to us. However, many published regional surveys include sites potentially impacted by mines or by metals from other sources (Section 3.5).

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The AQUAMIN supporting documents indicated that FCS were conducted less frequently than surveys of tissue residues in Canadian mine EEM programs. Data analyses were often qualitative and non-statistical. Individual species abundances were occasionally analyzed using univariate statistics; multivariate analyses were not conducted or at least were not reported by reviewers. None of the studies attempted to develop IBI-type composite indices. As expected, many communities, particularly in northern regions, contained few species.

4.3.4 Evaluation

4.3.4.1 Overall

The AETE screens monitoring tools based on two criteria:

- Has the method been successfully applied in the field?
- Is the method cost-effective?

FCS have been conducted successfully all over south and central North America, but studies of northern (boreal to Arctic) regions are rare. In these northern areas, depauperate fish communities may severely limit FCS (Section 4.3.4.2). The AETE will be better able to judge whether FCS are suitable for northern mines after 1997 field studies have been completed. In principle, an FCS should be successful and feasible at any site in south and possibly central Canada if communities of small fish in streams or lake littoral areas are sampled. Given the lack of success in capturing enough fish of a single species in the pulp and paper EEM AFS, definitions of what constitutes "reasonable effort" must be expanded if FCS are to be successful in a metal mine monitoring program. The AETE could contact individual U.S. mine sites to obtain a better idea of the suitability and cost-effectiveness of FCS for monitoring mines specifically. However, the AETE might also consider that most FCS are regional simply because this tool is more suitable on a regional level (see below).

FCS obviously require much greater sampling effort and field labour than AFS. Once the fish have been collected, processing costs are minimal unless internal exposure indicators are measured. As discussed elsewhere, the whole issue of why internal exposure indicators are important in AFS, but not FCS, needs to be resolved before costs of AFS versus FCS can be compared. Logically, internal exposure indicators should be measured in both types of survey, in which case analytical costs will be much greater for FCS, or in neither, in which case FCS are still more costly because of the greater sampling effort required. We suspect that internal exposure indicators are not considered important in most FCS because these surveys are conducted over broad spatial scales (i.e., regionally), often with many different point and non-point sources present.

Based on the above, FCS are likely to be too costly and/or too impractical to use at every mine site in a national program. There may be specific sites at which FCS may be more cost-effective than AFS, and a mandated national metal mine monitoring program should be sufficiently flexible to allow FCS to replace AFS at those sites. However, many of the disadvantages of FCS are substantially reduced when these surveys are conducted at a regional level. Costs can be shared among many industries and agencies, and costly analyses of internal exposure indicators would be unnecessary. In an environmental monitoring program, one exposed and one reference area could be surveyed at each mine site, and any assessment and analysis done on a regional or national basis (i.e., by comparing reference minus exposed among similar mine sites, with the sites as replicates). The AETE should at least consider this approach, although regional FCS may be more appropriate for provincial or watershed programs which include other industries as well as mines.

4.3.4.2 Specific Approaches

Fausch *et al.* (1990) review and critique the four types of FCS approaches discussed in Section 4.3.1 in detail. Table 3 summarizes their evaluation of the advantages and disadvantages of each approach, with our additions in italics.

Indicator taxa/guild surveys are relatively simple and easy to apply, especially on a regional basis where presence/absence data can be used. However, the best taxa or guilds to use may not be obvious *a priori*. The information provided would be limited unless the surveys were comprehensive and quantitative (in which case, why not use a multivariate or IBI approach?). Even if suitable indicator taxa were obvious *a priori*, the survey would be a population-level survey (direct or indirect) with the added problem that many sensitive taxa would be rare in both reference and exposed areas. In more comprehensive and quantitative surveys, indicator taxa and guilds can be useful *a posteriori* for interpretation. For example, if deposited solids were a concern, abundances of lithophils (i.e., species spawning on or in gravel) could be compared with abundances of other reproductive guilds which were not dependent on gravel for spawning.

Fausch *et al.* (1990) provide sound reasons for rejecting simple composite indices such as diversity or evenness, and we agree with them. Therefore, Table 3 evaluates only richness (number of taxa or S). S is perhaps the simplest index which can be calculated in community surveys, and it is one measure of biodiversity, which is obviously societally important. Theoretically, S can be calculated and analyzed for samples with few fish, which may not be suitable for analysis of abundance or CPUE (i.e., N). However, in practice, S is usually correlated with N at low N and both should be included in analysis (i.e., comparison of S-N regressions among areas). S is also dependent on stream order or size (Karr *et al.*, 1986; Fausch *et al.*, 1990) and lake area (Rago and Wiener, 1986). These correlations are probably not an issue in small-scale surveys within relatively homogeneous streams or lakes. However, the correlations can confound regional surveys. Because S is an important but limited variable, it should be incorporated into more quantitative and comprehensive surveys, but probably should not be used as a stand-alone variable.

Multivariate approaches are commonly used in macroinvertebrate community surveys, and can be used on either qualitative (presence/absence) or quantitative (abundance or CPUE) data. Multivariate analyses offer quantitative sophistication, even when qualitative data are analyzed. They are flexible and widely applicable, and have been used to analyze many types of data other than community data. Multivariate analyses integrate information from many variables (usually species), and are more objective than IBI approaches because no *a priori* decisions are made about which variables or species are most important. However, multivariate analyses are complex and results may be difficult to interpret. There are numerous procedures available, and it is difficult to choose the best and to standardize approaches in national programs. Many multivariate analyses require large sample sizes, especially if many variables are measured. The standard rule is to use at least 5-10 times as many replicates as variables (i.e., species or higher-level taxa) (Green, 1979; Tabachnick and Fidell, 1989). The results of multivariate analyses are usually site-specific and difficult to apply to other sites. For example, results from an ordination of communities at one mine site will rarely be similar in details to results from another site. Therefore, one of the best uses of multivariate analyses is to provide an objective description of community patterns which will then be used to develop simpler summary indices (e.g., IBI) for presentation and possibly management.

IBI and related indices integrate structure and function, and can be biologically meaningful. The basic approach is flexible and widely applicable, but individual metrics must be developed for each region. The metrics used are responsive to different sources of environmental degradation, which may not be an advantage in monitoring single sources, but is an advantage for regional programs. IBI scores are robust and reproducible, which may not be the case for results from more complex multivariate analyses. IBI and other indices are commonly used for management.

IBI and other indices are rarely suitable for communities with few species, because the metrics usually depend on contrasting abundances of different groups of species. For example, it is difficult to see how IBI could be developed for some of the depauperate communities surveyed in previous AETE studies (e.g., EVS, ESP and JWEL, 1996). IBI was developed in the midwestern U.S., where there are many more species than in Canadian drainages. For example, Page (1984) lists 71 described species of darters in Tennessee and 22 in Ohio, but only 12 in Ontario (mostly southern Ontario), 7 in Quebec, 5 in Manitoba, 4 in Saskatchewan, 1 in Alberta, and none in B.C., the Maritimes, and the Northwest Territories. Furthermore, the species present in Canada are probably the most widespread and tolerant; IBI developed in the U.S. rely heavily on the assumption that darters are more sensitive than other fish species.

Development of an IBI requires background ecological information, usually based on samples from many sites. Development of an IBI for a specific mine site would be a circular and senseless exercise, since metrics would be based largely on the variables which differed most between reference and exposed areas. It would hardly be surprising then to find significant differences in the IBI between areas. IBI should be based on regional surveys, and only then applied to individual sites.

IBI are composite indices, combining potentially unlike and uncorrelated variables using an arbitrary weighting. Composite indices may be unsuitable for statistical analysis (Green, 1979) and may conceal interesting patterns which would be revealed by multivariate analyses. Suter (1993) provides other criticisms of IBI and related indices, and concluded that an AFS approach was probably superior for EEM.

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Based on the above, more comprehensive and quantitative surveys using multivariate analyses and/or IBI-type indices are preferable to simpler approaches relying on indicator taxa or guilds, or on richness. However, the latter can be an important component of the former. Multivariate analyses are likely to be more suitable than IBI at individual sites, and should always be used to objectively verify the patterns which are assumed to exist when IBI are developed. Unless the AETE is prepared to commit to a regional approach in metal mine monitoring, they should accept that IBI cannot be used as in the U.S., and should not treat FCS and IBI as synonymous.

4.3.5 Recommendations

Fish community surveys (FCS) will rarely be practical and cost-effective for monitoring at individual mine sites and are not recommended for general use in a metal mine monitoring program. However, any national environmental monitoring program should be flexible enough to allow FCS to replace AFS at the few sites where FCS are practical and cost-effective. The FCS has been used extensively and successfully for monitoring and managing environmental quality on a regional basis in the U.S. FCS are recommended for use in regional programs in Canada, although a national metal mine monitoring program may not be the best vehicle for regional assessment.

5.0
COMPARATIVE EVALUATION OF TOOLS AND RECOMMENDATIONS

Evaluations of the suitability of the reviewed methods for assessing effects of mining activities (i.e., the discharging of metal-contaminated and possibly low-pH effluents to the receiving environment) on fishes are summarized in Tables 4 and 5. Although the methods are divided into two tables (because not all evaluation categories were applicable to both sub-organism-level methods and organism and higher - level methods), the same scales were used to rank variables for categories in common.

Sub-organism-level assessment methods (Table 4) for fishes were ranked or rated based on:

- Relevance: as an indicator of exposure to metals and low pH, and as a predictor of effects at higher (and more ecologically important) levels of biological organization.
- Specificity to metals and low pH.
- Response time.
- Difficulty of application in a monitoring program: in terms of technical and logistical considerations, and cost or effort required for sample collection, processing and analysis.
- Accessibility or commercial availability.

Organism and higher-level fish variables (Table 5) were ranked or rated based on:

- Relevance.
- Sensitivity, to metals and low pH, and to other stressors.
- Specificity to metals and low pH.
- Response time.
- Factors affecting cost: required sampling frequency, field collection costs, sample processing costs.
- Accessibility or commercial availability.

Relevance of variables was considered in terms of (a) the degree to which they indicate a fish's exposure to mining-related contaminants, and (b) their ecological importance (i.e., their bearing on fish survival and reproduction). Organism and higher-level fish variables were not ranked for quality as an indicator of exposure because this criterion would not likely be used to justify the inclusion of these variables in a monitoring program. Ecological relevance of variables included their importance for protecting aquatic biota. Some variables or tools (e.g., tissue metals in sport or commercial fish) may also be relevant for protection of human health. Only survival, growth, reproduction, and population- or community-level variables or tools, were considered highly relevant. Sub-organism measures were considered somewhat relevant only if obviously or strongly linked to highly relevant variables.

Tools that are sensitive and specific to metals and low pH will be most useful where chemical contamination of effluent discharges, leachate and fine tailings is the major concern. These tools will rarely be useful where physical habitat alterations associated with discharge of tailings, placer mining, mine site construction and development, and road construction, are the major concerns. Non-specific, sensitive tools will be the most useful tools where the primary stressors of concern are unknown, or are both chemical and physical.

Intermediate response times (i.e., effects or changes evident within several months to a year) are probably optimal for mine EEM programs conducted annually or less frequently. Most variables with rapid response times (within days or weeks) change too frequently to provide evidence of long-term or persistent effects from a single sampling trip. For these variables, the frequency and transience, rather than magnitude, of effects are the important issues. Consequently, frequent sampling would be required for an accurate description of stressor-related alterations. Effects on variables with response times >>1 year may not be evident within the lifetime of many mines. If effects occurred, timely remediation or mitigation might be impossible to implement.

The technical/logistical difficulty of measuring a variable (Table 4) refers to the collection and handling of a sufficient number of samples in a suitable condition for laboratory analyses. It is assumed the measurements of most sub-organism-level variables will be performed in specialized laboratories.

Variables that must be sampled bimonthly or more frequently will be costly, especially at remote sites. The most useful variables will be those that can be sampled annually or less frequently. Field collection costs are largely associated with collection time (i.e., field labour costs). Labour costs and time will depend on the number of sites sampled, and the number of fish required per site. Processing costs include labour and equipment costs for sample processing in the field and laboratory. Low-cost variables such as species identifications and counts, weights and lengths can be quickly measured in the field with inexpensive equipment and without dissecting the fish. Some variables requiring dissection (e.g., testes or liver weights) can also be measured in the field. Shipping, storage and sometimes sample preservation costs will be incurred for any variables requiring laboratory processing. Some variables, such as fecundity or age, can be determined in the laboratory without specialized equipment. Other variables, such as biochemical indicators and tissue metals require considerable sample preparation and expensive analytical equipment.

Tables 4 and 5 assume that the cost of collection or sampling equipment will be similar for all fish variables. However, the AETE should assume that sampling gear for fish is likely to be more expensive than sampling gear for collecting algae, invertebrates, and water column or sediment samples.

Variables or tools used in any national EEM program should be accessible or commercially available to consultants conducting the studies. In other words, the consultants should be able to make the required measurements in the field or in their own laboratories, or ship samples to nearby analytical laboratories with the appropriate capabilities.

5.1 EVALUATION OF TOOLS

It has long been recognized that the most meaningful assessments of ecological effects of environmental disturbances involve determining changes in the sizes of the constituent populations of "valued" species (Beanlands and Duinker, 1983). Effects at lower levels of biological organization are not important unless they ultimately alter populations. Evaluations of populations, in turn, allow assessments of effects on communities and whole ecosystems. Sub-organism ("lower-level") variables are relevant in monitoring programs only to the degree that they predict damage to population (i.e., are "early indicators"), or aid in establishing causal relationships between environmental disturbances and the ecologically important observations.

Growth, reproduction, population and community variables were defined *a priori* as highly relevant because of their ecological importance. Required measurements for these tools are easily made in the field or laboratory by B.Sc. and community-college technical personnel. Age determinations and species identifications should be verified externally by one or more experts. Among the chemical and biomarker tools, only biochemical indicators of growth and reproduction were potentially relevant. However, because of their comparative cost and technical difficulty they would not likely be preferable to direct measurements of fish growth and reproduction.

No organism or higher-level fish variable will be specific to metals and low pH (Table 4). All of these variables have been successfully used to monitor effects from many different stressors and anthropogenic activities. As noted elsewhere, the lack of specificity is only a disadvantage if:

- Direct effects from chemical contamination are the primary concern.
- Effects from other factors (i.e., nuisance variables) are poorly controlled in study designs.

We remain unconvinced that mine EEM programs should focus exclusively, or even predominantly, on the direct effects of chemical stressors. We also recommend that removal of nuisance variance be considered an important objective in the development and evaluation of mine EEM study designs.

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Because higher-level fish variables are non-specific, some lower-level exposure variable or indicator will generally be useful in any fish survey. The alternative is to assume that fish are long-term residents of sites in which they are captured. That assumption may be reasonable for smaller, less mobile fish, and must be made where physical rather than chemical stressors are the major concern. However, whenever possible, chemical exposure indicators or tracers, such as concentrations of metals in fish tissues, should be measured in fish surveys. Even if effects from chemical contamination are a minor concern, chemical or biochemical tracers provide some information on residency and exposure history. Unfortunately, most of the biochemical- and tissue-level methods reviewed for this report were not sufficiently specific for conditions of elevated metals or low pH. In addition, because of their rapid response times and/or difficulties in application, most of the biomarker methods are not presently suitable for inclusion in an EEM program for mining activities, except perhaps in investigative studies aimed at establishing causal relationships between metal contaminants/low pH and observed higher-level effect.

The organism or higher-level variables or tools are all highly relevant (except condition) and non-specific, and accessible, but differ in other categories in Table 4. An AFS incorporating growth, reproduction, condition, and several other organism-level variables is likely to be the most effective tool for general application in a national program. An AFS will be:

- Ecologically relevant.
- Sensitive to chemical and non-chemical stressors.
- Responsive within the lifetime of most mines, and scheduling of an EEM program.
- Reasonably cost-effective.

An AFS provides integrative and diagnostic capabilities relative to measuring and analyzing one or a few component organism-level variables. In other words, population-level effects and potential causes can be inferred, following the framework in Gibbons and Munkittrick (1994). The extra cost associated with the integrated AFS approach versus measuring a subset of organism-level variables will be low whenever the costs of any sampling trip and the costs of collecting the fish are the major costs.

The primary limitation of direct population-level assessments using CPUE, and FCS, is the high cost of sampling the required multiple sites. These two tools should be used whenever those costs are low, because they are more relevant and direct assessments of effects than an AFS.

5.2 CONCLUSIONS

No EEM program will be relevant without a direct or indirect assessment of population- or community-level effects on resident biota.

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Concentrations of metals in tissues should be measured as a means of establishing exposure of mobile biota to metal-contaminated mining effluent.

We cannot conclude from this review that:

- Fish are necessarily more suitable than other organisms for monitoring higher-level effects.
- Lower-level tools or variables are necessarily irrelevant or unsuitable.
- The AFS, and specific target species (e.g., large versus small), will always be the "best" at any site.

Our review clearly indicated that:

- Environmental toxicologists have consistently ignored opportunities to assess or measure higher-level variables and effects at little or no cost when measuring lower-level effects and variables.
- Biochemical- and tissue-level effects are better suited for investigations of potential causes of higher-level effects rather than as monitoring variables.
- All tools or variables have their own purposes, advantages, and limitations.
- Both the pulp and paper EEM expert working groups and the AQUAMIN Final Report indicated that site-specific flexibility is required in any national program.

Site-specific flexibility will only be possible when the purposes of monitoring programs are clearly defined, and the advantages and limitations of alternative tools and variables are known and carefully considered. To meet that objective, general recommendations re: the most effective tools, and guidance as to where or when specific tools may or may not be suitable were provided in this report. ***No standard approach will be universally applicable. Specific recommendations will only restrict the range of suitable options, which will limit the effectiveness of any national EEM program and any assessments of effects on fish.***

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FIGURES

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Figure 1. Impact assessment framework (Munkittrick and Power, 1990).

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Figure 2. Ecological relevance and specificity of biological effects measurements (Addison, 1996).

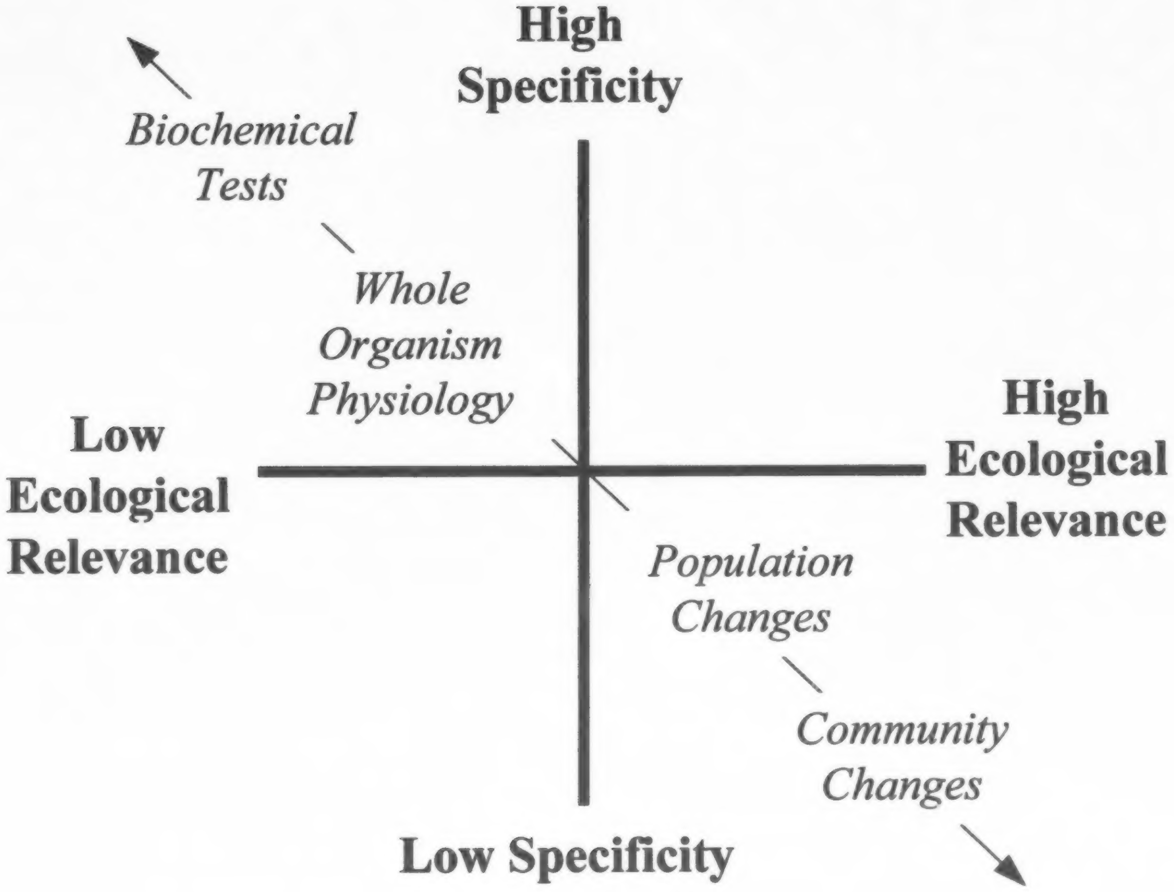

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Figure 3. Growth of unexposed mature adult white sucker (*Catostomus commersoni*) in an Alberta river receiving pulp mill discharge (EVS, 1996). Vertical bars are ± 1SD.

Tables

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Table 1. Summary evaluation of selected sub-organism-level biomarkers reviewed by Munkittrick and Power (1990).

PARAMETER	FUNCTION	EFFECT OF METALS	CONFOUNDING FACTORS	CHARACTERISTICS	ADVANTAGES	DISADVANTAGES
DIRECT CHANGES						
delta Aminolevulinic Acid Dehydratase (ALAD) enzyme activity	An important enzyme governing hemoglobin synthesis	Only lead (Pb) is inhibitory *in vivo*; Near lethal levels of Cu, Hg, Ag, Cd, Zn did not inhibit ALAD activity	ALAD may not be sensitive to alkylated forms of Pb; Zn may be a co-contaminant	Pb inhibition of ALAD occurs in spleen, kidney, liver; blood is most convenient tissue to assay	ALAD activity generally decreases with [Pb], good correlation with blood; level provides early warning of neurotoxicity; good indicator of acid mine drainage	Biotic and environmental factors affect bioavailability of Pb; poor relationship between ALAD and fish performance; inhibition better used as indicator of exposure rather than toxic response; ALAD varies with assay, temperature, substrate, pH, Zn, species
Cholinesterase Enzymes	Functions to terminate effects of neurotransmitter in muscle, brain; regulate ionic permeability of cell membranes in tissues	Activity may be inhibited by metals at high concentrations	Organophosphate and carbamate insecticides produce same effect	Enzyme inhibition in brain and skeletal tissue	Tissue samples are easy to collect; time frame for exposure is very quick (minutes); tissues can be stored frozen for a long period of time with little loss of activity	Enzyme activity can be affected by assay technique, growth, age, reproductive state, sex; poor relationship between enzyme level and growth, survival, feeding. Large sample size required; Use of these enzymes for metal exposure poorly dev
Metallothionein (MT)	Regulation of essential metals (Cu, Zn); detoxification of metals; acquisition of metal tolerance	Induction (synthesis) of MT binding of metals (Hg, Cd, Zn, Cu, Ag)	Reproductive condition, cellular metal requirements, capture stress, ambient water temperature	Binding and sequestering of metals (Cu, Zn, Cd); accumulation of metal-MT in lysosomes	Dose-dependent response to ambient metal concentrations; high MT levels associated with detrimental organism- and population- level effects; reliable MT quantification methods exist	Non-toxicological factors affecting MT levels require assessment to interpret MT responses
INDIRECT CHANGES						
Corticosteroid Proteins	Any factor that causes stress (handling, crowding, shock, hypoxia, toxicant exposure, disease) causes release of corticosteroids	Increase in production and release of corticosteroids and catecholamines which can be quantified according to degree of stress	Any perceived stressor (disease, sampling, other toxicants) can afect protein levels	Degree of impact quantified by increase in plasma corticosteroid (short-term) or inteerregnal nuclear diameter (long-term)	Integrate over stressors and time, and can be used to assess population level effect;	Exposure to many factors excluding metals can cause effect; high variability; data difficult to interpret little ability to relate to changes in indicators to population level

Table 1 (continued)

Parameter	Function	Effect of Metals	Confounding Factors	Characteristics	Advantages	Disadvantages
Brain Neuroamines	Dopamine, noradrenalin, serotonin regulate many physiological functions (hormone release, thermoregulatory behavior)	Organic toxicants change brain neuroamine levels causing motor and swim bladder dysfunction	Whole brain amine status has been used to demonstrate exposure to PCBs, PAH, DDT, pesticides	Changes in whole brain neuroamine concentration, physical performance	Gross changes reflect exposure to organic chemicals; whole brain concentrations may have general values indicator of contaminant exposure	Impractical to collect microscopic areas of fish brain; changes in whole brain levels inconclusive; unclear relationship with changes in physical performance; levels affected by season, reproductive state
Hepatic Mixed Function Oxidase (MFO)	Large group of enzymes that metabolize lipophilic, poorly excreted compounds to more easily excreted, hydrophillic compounds	Metal exposure not found to induce MFO activity	MFO enzymes induced by petroleum hydrocarbons, sewage, pollution; Activity varies with gender, age, liver size, species, diet	Rapid induction (peak within 96 h of exposure); Activity declines rapidly (7-10 d) after exposure is removed	Easy to measure; rapid response; variability can be reduced with appropriate statistical design	No method available to estimate accuracy or precision of results; large sample size required; although induction is dose dependent, dose response curves unavailable; saturation of receptor sites
Brain Monoamine Oxidase (MAO)	Metabolizes biogenic amines, transmitters that are used to coordinate physiological processes	Inhibition of brain MAO may cause altered metabolism, changes in amines and indoleamines in neural tissue, possible delay in maturation	Little work done on fish	Heavy metals inhibit the enzymes activity	Established relationship between reduction in brain MAO and exposure to mercuric substances	Few studies done; no good relationship between metals exposure and brain MAO activity
ATPase Activity	Essential enzyme involved in transporting electrolytes across cell membranes and ionic regulation	Reduction in activity causing ionic imbalance, disruption of brain synaptic transmission	ATPase activity is not a useful measure of sublethal toxicity in absence of other factors	May affect ion and water balance of estuarine fish, smolts	Gill activity a useful indicator of acid stress; may serve as indicator of metals impact on metabolism	Response of gill tissue to exposure can vary with developmental stage; should be combined with other measures
Leucine Aminonapthyl-amidase (LAN) Activity	A proteolytic enzyme contained within lysozymes	Metals are known to cause lysozyme membranes to rupture, releasing LAN into the cytosol	Other elements (free radicals, peroxides, PAHs, ionizing radiation) cause lysozyme rupture	Increase in LAN concentration in fish plasma may be good indicator of cell death	Concentration of LAN may be related to metal exposure; levels are unaffected by handling, electrofishing, blood sampling	Levels may increase with fish age; LAN may be unstable and results highly variable; No field trials conducted; rapid sampling of large numbers of fish required
Sorbitol Dehydrogenase (SSDH)	Catalyzes reversible oxidation-reduction reactions in the liver	Exposure to waterborne metals (e.g. Cu) cause increase in SSDH	Wide number of organic chemicals also produce elevated levels	Liver specific reaction	Response is quicker, and at lower level of exposure than LSI or histological changes	An increase in SSDH has not been correlated with meaningful effect at higher levels of organization
Transaminase Enzymes	A class of enzymes that detoxifies ammonia in liver; indicator of liver or kidney damage in mammals	Levels are correlated with exposure to toxicants	Contamination of reagent kits or blood with high ammonia confound results; other types of liver damage cause similar results	High levels in plasma ammonia is indicator	Enzymes have been correlated with exposure to toxicants	No changes associated with higher levels of organization; very limited field study; other factors can cause same efect

Table 1 (continued)

Table 2. Summary evaluation of organism- and higher-level monitoring tools. (See Section 3.3, 3.4 and 3.5 for details.)

PARAMETER	FUNCTION	EFFECT OF METALS/LOW PH	CONFOUNDING FACTORS	CHARACTERISTICS	ADVANTAGES	DISADVANTAGES
ORGANISM						
Growth	Change in body size over time; body size is related to most biological variables	Usually -ve; variable at low metal levels and intermediate pH	Many natural and anthropogenic factors; reproductive effort	Reduced size-at-age	Integrates over time; easily measured	Individual growth may be difficult to estimate; response not rapid except in younger fish
Reproductive effort	Production of gametes; measure of number of young produced	Usually -ve	Many natural and anthropogenic factors; growth	Reduced gonad weight or delayed maturity	More rapid response than growth; easily measured	Requires mature adults; viability of later stages not easily estimated from number of ova
Condition	Robustness or "fatness"; index of "well-being"	Not well known	Many natural and anthropogenic factors	Fish with poor condition will be emaciated	Precise and economical; rapid response	Can be transient; less relevant than growth or reproduction
POPULATION						
Direct (CPUE)	Estimates abundance or biomass	Usually -ve; varies among species	Many natural and anthropogenic factors, esp. exploitation	Decline in abundance/catch	Relevant biologically and societally	High sampling effort; estimates imprecise
Indirect (AFS)	Measures organism-level variables which determine population growth	Usually -ve for component variables	Many natural and anthropogenic factors	Response patterns depend on life stage and processes affected	Low sampling effort; integrates organism-level effects; can identify indirect effects (i.e., on prey)	Qualitative; interpretation may be difficult
COMMUNITY						
Fish community survey (FCS)	Measures number of species; relationships among them	Depends on life stages, species affected	Many natural and anthropogenic factors	Tolerant spp. replace sensitive spp. (low pH; metals?); richness declines	Relevant biologically and societally; useful for regional monitoring	High sampling effort; may not be useful for point-source monitoring

Table 3. Evaluation of fish community survey approaches. (From Table 3 in Fausch *et al.*, [1990] with our additions in italics.)

APPROACH	ADVANTAGES	DISADVANTAGES
Indicator taxa/guilds	(1) simple; easily applied (2) guilds allow resolution of specific stresses (3) *useful for interpreting more comprehensive surveys*	(1) best taxa/guilds may not be obvious; may vary spatially, temporally and among stressors (2) taxa/guilds may be affected by other species present (3) conveys little information without more comprehensive or quantitative sampling (4) *indicator taxa may be rare in all areas and difficult to collect*
Richness (*S*)	(1) simple; easily applied (2) *societally important; measure of biodiversity* (3) *necessary component of more comprehensive surveys*	(1) dependent on abundance, *stream order, lake area* (2) necessary, but not sufficient
Multivariate	(1) quantitative, even for presence/absence data (2) *integrative*; simultaneously compares all samples, *species* (3) *flexible and universally applicable*; can compare abundance or biomass, structure or function (4) *objective/empirical*	(1) complex; difficult to interpret (2) many methods; best method rarely obvious (3) *may not be robust or repeatable*; statistical properties of some methods poorly known (4) *may require large sample sizes (many more replicates than species)*
Index of Biotic Integrity (IBI) and related indices	(1) integrates structure and function (2) biologically meaningful (3) flexible and widely applicable *(??)* (4) robust and repeatable (5) metrics sensitive to different stressors *(?? see Disadvantages)* (6) *suitable for management*	(1) requires at least moderate species richness (2) requires background ecological information; *often many reference samples* (3) partly subjective (4) metrics must be modified for each region (5) biomass not included *(it could be)* (6) statistical properties not well studied (7) *compositing potentially unlike and uncorrelated metrics may cause problems for analysis and interpretation* (8) *more suitable for regional, rather than point-source, monitoring*

Table 4. Evaluation of sub-organism-level methods for assessing effects of mining effluent on fishes in monitoring programs.

VARIABLE/TOOL	RELEVANCE		SPECIFICITY TO METALS, LOW PH	RESPONSE TIME	DIFFICULTY OF APPLICATION		AVAILABILITY
	INDICATIVE OF EXPOSURE	PREDICTIVE OF ECOLOGICAL EFFECTS			TECHNICAL/ LOGISTICAL	COST / EFFORT	
CHEMICAL-LEVEL							
Metal concentrations in tissues	1	2	1	Int.	Low	Med.	1
BIOCHEMICAL-LEVEL							
Ion status	2	2	2	Fast	High	Med.	3
Inhibitory/inducible enzymes and other proteins	2	3	1-3	Fast	Med.	Med.	2?
Metallothionein[1]	1	1?	1	Fast	Med.	Med.	2-3
Lipids/carbohydrates	2	2	3	Int. - Fast	Low	High	2
Indicators of growth	2	1	3	Int.	Med.	Med.	3
Indicators of reproduction	3	2	3	Int. - Fast	High	Med.	2
Indicators of immunity	3	3	3	Fast	High	High	2
Indicators of respiration	2	3	2	Fast	High	High	3(?)
Genotoxic effects	3	3	3	Slow - Int.	Med.	High	3
TISSUE-LEVEL							
Organ pathology	2	2	2	Slow - Int.	Low	Med.	2
Histopathology[1]	2	2	2	Int.	High	Med. - High	

SCALES:
Relevance 1 = highly relevant for assessing effects of metals, low pH; 2=somewhat relevant; 3=not very relevant (or lack of data indicating relevance)
Specificity 1 = highly specific; 2=somewhat specific; 3=non-specific
Response time Fast = within days or weeks; Int.=within months or a year; Slow=over several years

Table 4 (continued)

Difficulty of Application:	Technical/logistical	Low = minimal skill and judgement required for collection of samples for submission to laboratory ; Med. = moderate amount of skill and judgement required; High = specialized training and/or professional judgement required
	Cost /effort	Low = sample collection and/or laboratory analyses relatively inexpensive; Med. = sample collection and/or laboratory analyses moderate in cost/effort; high = sample collection and/or laboratory analyses expensive/time consuming
Availability		1 = required services/analyses widely available; 2 = required services/analyses provided by some consultants/laboratories; 3 = required services/analyses provided by few consultants/laboratories

1 Metallothionein and histopathology were not reviewed in detail in the report. Key findings of separate reports prepared for AETE on these subjects are included in this table for completeness.

Table 5. Evaluation of organism- and higher- level methods for assessing effects of mining effluents on fishes in monitoring programs.

VARIABLE/ TOOL	RELEVANCE (ECOLOGICAL)	SENSITIVITY TO:		SPECIFICITY TO METALS, LOW PH	RESPONS E TIME	COST			AVAILABILITY
		METALS, LOW PH	OTHER STRESSORS			FREQUENCY	FIELD COLLECTION	PROCESSING	
ORGANISM									
Growth	1	1	1	3	Slow	Low	Med.	Low-Med.	1
Reproduction	1	1	1	3	Int.	Low	Med.	Low-Med.	1
Condition	2	2?	2?	3	Int.-Fast	Low	Med.	Low	1
POPULATION									
Direct (CPUE)	1	35796	35796	3	Slow	Low	High	Low	1
Indirect (AFS)	1	1	1	3	Int.-Slow	Low	Med.	Med.	1
COMMUNITY									
Community survey (FCS)	1	35796	1	3	Slow	Low	High	Low	1

NOTES: Int.=Intermediate; Med.=Medium

SCALES:

Relevance (ecological) 1 = highly relevant; 2 = somewhat relevant; 3 = not very relevant

Sensitivity 1 = very sensitive; 2 = somewhat sensitive; 3 = not very sensitive

Specificity 1 = highly specific; 2 = somewhat specific; 3 = non-specific

Response time Fast = within days or weeks; Int. = within months or a year; Slow = over several years

Cost:

Frequency (of sampling) Low = annual or less frequent; Med. = 1-5 times per year; High = >5 times per year

Field Collection Low = <20 fish/site, few sites required; Med. = ≥20 fish/site; few sites;≥20 fish/site; multiple sites

Processing (in field and lab) Low = a few simple measurements, mostly in the field, required; Med. = some simple lab measurements or processing required; High = expensive field or lab processing, equipment required

Availability 1 = required services/analyses widely available; 2 = required services/analyses provided by some consultants/laboratories; 3 = required services/analyses provided by few consultants/laboratories